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18431 AND 32374, NOVEL HUMAN PROTEIN KINASE FAMILY MEMBERS AND USES THEREFOR

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Background of the Invention

Phosphate tightly associated with protein has been known since the late nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins have been found. The most common involve esterification of phosphate to serine, threonine, and tyrosine with smaller amounts being linked to lysine, arginine, histidine, aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated proteins implies the existence of one or more protein kinase capable of phosphorylating amino acid residues on proteins, and also of protein phosphatases capable of hydrolyzing phosphorylated amino acid residues on proteins.

Kinases play a critical role in the mechanism of intracellular signal transduction. They act on the hydroxyamino acids of target proteins to catalyze the transfer of a high energy phosphate group from adenosine triphosphate (ATP). This process is known as protein phosphorylation. Along with phosphatases, which remove phosphates from phosphorylated proteins, kinases participate in reversible protein phosphorylation. Reversible phosphorylation acts as the main strategy for regulating protein activity in eukaryotic cells.

Protein kinases play critical roles in the regulation of biochemical and morphological changes associated with cell proliferation, differentiation, growth and division (D'Urso, G. *et al.* (1990) *Science* 250: 786-791; Birchmeier, C. *et al.* (1993) *Bioessays* 15: 185-189). They serve as growth factor receptors and signal transducers and have been implicated in cellular transformation and malignancy (Hunter, T. *et al.* (1992) *Cell* 70: 375-387; Posada, J. *et al.* (1992) *Mol. Biol. Cell* 3: 583-592; Hunter, T. *et al.* (1994) *Cell* 79: 573-582). For example, protein kinases have been shown to participate in the transmission of signals from growth-factor receptors (Sturgill, T. W. *et al.* (1988) *Nature* 344: 715-718; Gomez, N. *et al.* (1991) *Nature* 353: 170-173), control of entry of cells into mitosis (Nurse, P. (1990) *Nature* 344: 503-508; Maller, J. L. (1991) *Curr. Opin.*

Cell Biol. 3: 269-275) and regulation of actin bundling (Husain-Chishti, A. *et al.* (1988) *Nature* 334: 718-721).

Kinases vary widely in their selectivity and specificity of target proteins. They still may, however, comprise the largest known enzyme superfamily. Protein kinases can be divided into two main groups based on either amino acid sequence similarity or specificity for either serine/threonine or tyrosine residues. Serine/threonine specific kinases are often referred to as STKs while tyrosine specific kinases are referred to as PTKs. A small number of dual-specificity kinases are structurally like the serine/threonine-specific group. Within the broad classification, kinases can be further sub-divided into families whose members share a higher degree of catalytic domain amino acid sequence identity and also have similar biochemical properties. Most protein kinase family members also share structural features outside the kinase domain that reflect their particular cellular roles. These include regulatory domains that control kinase activity or interaction with other proteins (Hanks, S.K. *et al.* (1988) *Science* 241: 42-52).

Almost all kinases contain a catalytic domain composed of 250-300 conserved amino acids. This catalytic domain may be viewed as composed of 11 subdomains. Some of these subdomains apparently contain distinct amino acid motifs which confer specificity as a STK or PTK or both. Kinases may also contain additional amino acid sequences, usually between 5 and 100 residues, flanking or occurring within the catalytic domain. These residues apparently act to regulate kinase activity and to determine substrate specificity. (Reviewed in Hardie, G. and Hanks, S. (1995) *The Protein Kinase Facts Book*, Vol I:7-20 Academic Press, San Diego, Calif.)

Approximately one third of the known oncogenes encode PTKs. PTKs may occur as either transmembrane or soluble proteins. Transmembrane PTKs act as receptors for many growth factors. Interaction of a growth factor to its cognate receptor initiates the phosphorylation of specific tyrosine residues in the receptor itself as well as in certain second messenger proteins. Growth factors found to associate with such PTK receptors include epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, hepatocyte growth factor, insulin and insulin-like growth factors, nerve growth factor, vascular endothelial growth factor, and macrophage colony stimulating factor.

Soluble PTKs often interact with the cytosolic domains of plasma membrane receptors. Receptors that signal through such PTKs include cytokine, hormone, and

antigen-specific lymphocytic receptors. Many PTKs were identified as oncogene products by the observation that PTK activation was no longer subject to normal cellular controls. Also, increased tyrosine phosphorylation activity is often observed in cellular transformation, or oncogenesis, (Carbonneau, H. and Tonks, N. K. (1992) Annu. Rev. Cell Biol. 8:463-93.) PTK regulation may therefore be an important strategy in controlling some types of cancer.

Summary of the Invention

The present invention is based, in part, on the discovery of novel human protein kinase family members, referred to herein as "32374" or "18431." The nucleotide sequence of a cDNA encoding 32374 or 18431 is shown in SEQ ID NO:1 or SEQ ID NO:4, and the amino acid sequence of a 32374 or 18431 polypeptide is shown in SEQ ID NO:2 or SEQ ID NO:5. In addition, the nucleotide sequence of the coding region is depicted in SEQ ID NO:3 or SEQ ID NO:6.

Accordingly, in one aspect, the invention features a nucleic acid molecules which encode a 32374 or 18431 protein or polypeptide, e.g., a biologically active portion of the 32374 or 18431 protein. In a preferred embodiment, the isolated nucleic acid molecules encode polypeptides having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5. In other embodiments, the invention provides an isolated 32374 or 18431 nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____. In still other embodiments, the invention provides nucleic acid molecules that are sufficiently or substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____, wherein the nucleic acid encodes a full length 32374 or 18431 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include a 32374 or 18431 nucleic acid molecule described herein. In certain embodiments,

the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 32374 or 18431 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing 32374 or 18431 nucleic acid molecules and polypeptides.

5 In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 32374- or 18431-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a 32374 or 18431 encoding nucleic acid molecule are provided.

10 In another aspect, the invention features, 32374 or 18431 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of protein kinase family-associated or other 32374- or 18431-mediated or -related disorders. In another embodiment, the invention provides 32374 or 18431 polypeptides having a 32374 or 18431 activity. Preferred
15 polypeptides are 32374 or 18431 proteins including at least one protein kinase family domain, and, preferably, having a 32374 or 18431 activity, e.g., a 32374 or 18431 activity as described herein.

In other embodiments, the invention provides 32374 or 18431 polypeptides, e.g., a 32374 or 18431 polypeptide having the amino acid sequence shown in SEQ ID NO:2 or
20 SEQ ID NO:5; the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number ____; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number ____; or an amino acid sequence encoded by a nucleic acid molecule having a
25 nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____, wherein the nucleic acid encodes a full length 32374 or 18431 protein or an active fragment thereof.

30 In a related aspect, the invention further provides nucleic acid constructs which include a 32374 or 18431 nucleic acid molecule described herein.

In a related aspect, the invention provides 32374 or 18431 polypeptides or fragments operatively linked to non-32374 or -18431 polypeptides to form fusion proteins.

In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 32374 or 18431 polypeptides.

5 In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 32374 or 18431 polypeptides or nucleic acids.

In still another aspect, the invention provides a process for modulating 32374 or 18431 polypeptide or nucleic acid expression or activity, e.g. using the compounds identified in the screens described herein. In certain embodiments, the methods involve
10 treatment of conditions related to aberrant activity or expression of the 32374 or 18431 polypeptides or nucleic acids, such as conditions involving aberrant or deficient protein kinase function, cellular proliferation or differentiation, or pain.

The invention also provides assays for determining the activity of or the presence or absence of 32374 or 18431 polypeptides or nucleic acid molecules in a biological sample,
15 including for disease diagnosis.

In further aspect the invention provides assays for determining the presence or absence of a genetic alteration in a 32374 or 18431 polypeptide or nucleic acid molecule, including for disease diagnosis.

In another aspect, the invention features a two-dimensional array having a plurality
20 of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 32374 or 18431 molecule. In one embodiment, the capture probe is a nucleic acid, e.g., a probe complementary to a 32374 or 18431 nucleic acid
25 sequence. In another embodiment, the capture probe is a polypeptide, e.g., an antibody specific for 32374 or 18431 polypeptides. Also featured is a method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

Other features and advantages of the invention will be apparent from the following
30 detailed description, and from the claims.

Brief Description of the Drawings

Figure 1A-B depicts a cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human 32374. The methionine-initiated open reading frame of human 32374 (without the 5' and 3' untranslated regions) extends from nucleotide position 1 to position 1041 of SEQ ID NO:3, not including the terminal codon.

Figure 2 depicts a hydropathy plot of human 32374. Relatively hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The location of the transmembrane domains, and the extracellular and intracellular portions are also indicated. The numbers corresponding to the amino acid sequence of human 32374 are indicated. Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence, *e.g.*, a sequence above the dashed line, *e.g.*, the sequence from about amino acid 28 to 38, from about 160 to 170, and from about 290 to 305 of SEQ ID NO:2; all or part of a hydrophilic fragment, *e.g.*, a sequence below the dashed line, *e.g.*, the sequence from about amino acid 5-13, from about 245-255, and from about 320-330 of SEQ ID NO:2; a sequence which includes a Cys, or a glycosylation site.

Figure 3 depicts an alignment of the protein kinase domain of human 32374 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper sequence is the consensus amino acid sequence (SEQ ID NO:7), while the lower amino acid sequence corresponds to amino acids 1 to 231 of SEQ ID NO:2.

Figure 4 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain "kinase serine/threonine" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 59 of the 114 amino acid consensus sequence (SEQ ID NO:8), while the upper amino acid sequence corresponds to the "kinase serine/threonine" domain of human 32374, amino acid residues 226 to 286 of SEQ ID NO:2.

Figure 5 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain "kinase serine/threonine" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 89 to 114 of the 114 amino acid consensus sequence (SEQ ID NO:9), while the upper amino acid sequence corresponds to the "kinase serine/threonine" domain of human 32374, amino acid residues 321 to 346 of SEQ ID NO:2.

Figure 6 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain “kinase serine/threonine-protein X C01C4.3 chromosome ATP-binding transferase 2.7.1.” (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 3 to 82 of the 149 amino acid consensus sequence (SEQ ID NO:10), while the upper amino acid sequence corresponds to the “kinase serine/threonine-protein X C01C4.3 chromosome ATP-binding transferase 2.7.1.” domain of human 32374, amino acid residues 166 to 245 of SEQ ID NO:2.

Figure 7 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain “D2045.7” (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 16 to 128 of the 128 amino acid consensus sequence (SEQ ID NO:11), while the upper amino acid sequence corresponds to the “D2045.7” domain of human 32374, amino acid residues 4 to 118 of SEQ ID NO:2.

Figure 8 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain “F40A3.5” (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 175 to 316 of the 346 amino acid consensus sequence (SEQ ID NO:12), while the upper amino acid sequence corresponds to the “F40A3.5” domain of human 32374, amino acid residues 47 to 174 of SEQ ID NO:2.

Figure 9 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain “II EEED8.9 chromosome” (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 319 to 448 of the 758 amino acid consensus sequence (SEQ ID NO:13), while the upper amino acid sequence corresponds to the “II EEED8.9 chromosome” domain of human 32374, amino acid residues 77 to 202 of SEQ ID NO:2.

Figure 10 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain “Domain of unknown function” (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 647 to 842 of the 1557 amino acid consensus sequence (SEQ ID NO:14), while the upper amino acid sequence corresponds to the “Domain of unknown function” domain of human 32374, amino acid residues 68 to 263 of SEQ ID NO:2.

Figure 11 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain "Kinase CP0625 serine/threonine S/T TC0422 serine/threonine-protein" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 71 to 240 of the 381 amino acid consensus sequence (SEQ ID NO:15), while the upper amino acid sequence corresponds to the "Kinase CP0625 serine/threonine S/T TC0422 serine/threonine-protein" domain of human 32374, amino acid residues 30 to 189 of SEQ ID NO:2.

Figure 12 depicts a BLAST alignment of human 32374 with a consensus amino acid sequence derived from a ProDomain "Kinase kinase-related serine/threonine serine/threonine-protein" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 82 to 296 of the 542 amino acid consensus sequence (SEQ ID NO:16), while the upper amino acid sequence corresponds to the "Kinase kinase-related serine/threonine serine/threonine-protein" domain of human 32374, amino acid residues 29 to 262 of SEQ ID NO:2.

Figure 13A-D depicts a cDNA sequence (SEQ ID NO:4) and predicted amino acid sequence (SEQ ID NO:5) of human 18431. The methionine-initiated open reading frame of human 18431 (without the 5' and 3' untranslated regions) extends from nucleotide position 1 to position 2682 of SEQ ID NO:6, not including the terminal codon.

Figure 14 depicts a hydropathy plot of human 18431. Relatively hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The location of the transmembrane domains, and the extracellular and intracellular portions are also indicated. The numbers corresponding to the amino acid sequence of human 18431 are indicated. Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence, *e.g.*, a sequence above the dashed line, *e.g.*, the sequence from about amino acid 90 to 100, from about 472 to 482, and from about 620 to 630 of SEQ ID NO:5; all or part of a hydrophilic sequence, *e.g.*, a sequence below the dashed line, *e.g.*, the sequence from about amino acid 170 to 180, from about 360 to 370, and from about 710 to 720 of SEQ ID NO:5; a sequence which includes a Cys, or a glycosylation site.

Figure 15 depicts an alignment of the protein kinase domain of human 18431 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM.

The upper sequences are the consensus amino acid sequence (SEQ ID NO:17), while the lower amino acid sequences correspond to amino acids 43 to 273 of SEQ ID NO:5.

Figure 16 depicts an alignment of the TBC domain of human 18431 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM.

The upper sequences are the consensus amino acid sequence (SEQ ID NO:18), while the lower amino acid sequences correspond to amino acids 463 to 673 of SEQ ID NO:5.

Figure 17 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Cell division similar CG4041 pombe control L3169.1" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 170 of the 170 amino acid consensus sequence (SEQ ID NO:19), while the upper amino acid sequence corresponds to the "Cell division similar CG4041 pombe control L3169.1" domain of human 18431, amino acid residues 459 to 620 of SEQ ID NO:5.

Figure 18 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Cell division similar CG4041 pombe control" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 174 of the 174 amino acid consensus sequence (SEQ ID NO:20), while the upper amino acid sequence corresponds to the "Cell division similar CG4041 pombe control" domain of human 18431, amino acid residues 27 to 203 of SEQ ID NO:5.

Figure 19 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 100 to 230 of the 230 amino acid consensus sequence (SEQ ID NO:21), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 322 to 458 of SEQ ID NO:5.

Figure 20 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 4 to 73 of the 230 amino acid consensus sequence (SEQ ID NO:22), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 207 to 276 of SEQ ID NO:5.

Figure 21 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 99 to 107 of the 230 amino acid consensus sequence (SEQ ID NO:23), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 636 to 644 of SEQ ID NO:5.

Figure 22 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Chromosome FIS transmembrane cDNA similar frame reading ORF XV GTPase" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 87 of the 88 amino acid consensus sequence (SEQ ID NO:24), while the upper amino acid sequence corresponds to the "Chromosome FIS transmembrane cDNA similar frame reading ORF XV GTPase" domain of human 18431, amino acid residues 621 to 700 of SEQ ID NO:5.

Figure 23 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Kinase serine/threonine-protein transferase receptor ATP-binding 2.7.1.-tyrosine-protein phosphorylation precursor" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 136 to 220 of the 424 amino acid consensus sequence (SEQ ID NO:25), while the upper amino acid sequence corresponds to the "Kinase serine/threonine-protein transferase receptor ATP-binding 2.7.1.-tyrosine-protein phosphorylation precursor" domain of human 18431, amino acid residues 72 to 140 of SEQ ID NO:5.

Figure 24 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Kinase serine/threonine-protein transferase receptor ATP-binding 2.7.1.-tyrosine-protein phosphorylation precursor" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 245 to 377 of the 424 amino acid consensus sequence (SEQ ID NO:26), while the upper amino acid sequence corresponds to the "Kinase serine/threonine-protein transferase receptor ATP-binding 2.7.1.-tyrosine-protein phosphorylation precursor" domain of human 18431, amino acid residues 152 to 276 of SEQ ID NO:5.

Figure 25 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1;

<http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 29 to 141 of the 187 amino acid consensus sequence (SEQ ID NO:27), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 740 to 866 of SEQ ID NO:5.

5 *Figure 26 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 3 to 96 of the 187 amino acid consensus sequence (SEQ ID NO:28), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 693 to 776 of SEQ ID NO:5.*

10 *Figure 27 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "CG4041" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 152 to 186 of the 187 amino acid consensus sequence (SEQ ID NO:29), while the upper amino acid sequence corresponds to the "CG4041" domain of human 18431, amino acid residues 856 to 887 of SEQ ID NO:5.*

15 *Figure 28 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "FLJ11082 FIS place10005206 CG7742 cDNA" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 336 to 483 of the 531 amino acid consensus sequence (SEQ ID NO:30), while the upper amino acid sequence corresponds to the "FLJ11082 FIS place10005206 CG7742 cDNA" domain of human 18431, amino acid residues 506 to 652 of SEQ ID NO:5.*

20 *Figure 29 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Pro1038" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 5 to 139 of the 280 amino acid consensus sequence (SEQ ID NO:31), while the upper amino acid sequence corresponds to the "Pro1038" domain of human 18431, amino acid residues 77 to 213 of SEQ ID NO:5.*

25 *Figure 30 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "Pro1038" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 198 to 234 of the 280 amino acid consensus sequence (SEQ ID NO:32), while the upper amino*

acid sequence corresponds to the "Pro1038" domain of human 18431, amino acid residues 240 to 276 of SEQ ID NO:5.

Figure 31 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "FIS cDNA FLJ11046 CG4552 NT2RP4002052 FLJ10888 F20D1.2 place1004473" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 18 to 118 of the 556 amino acid consensus sequence (SEQ ID NO:33), while the upper amino acid sequence corresponds to the "FIS cDNA FLJ11046 CG4552 NT2RP4002052 FLJ10888 F20D1.2 place1004473" domain of human 18431, amino acid residues 596 to 691 of SEQ ID NO:5.

Figure 32 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "FIS cDNA FLJ11046 CG4552 NT2RP4002052 FLJ10888 F20D1.2 place1004473" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 194 to 214 of the 556 amino acid consensus sequence (SEQ ID NO:34), while the upper amino acid sequence corresponds to the "FIS cDNA FLJ11046 CG4552 NT2RP4002052 FLJ10888 F20D1.2 place1004473" domain of human 18431, amino acid residues 796 to 816 of SEQ ID NO:5.

Figure 33 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "SCY1 cDNA FIS kinase weakly serine/threonine-protein similar p23A10.10 for CG12524" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 160 of the 340 amino acid consensus sequence (SEQ ID NO:35), while the upper amino acid sequence corresponds to the "SCY1 cDNA FIS kinase weakly serine/threonine-protein similar p23A10.10 for CG12524" domain of human 18431, amino acid residues 112 to 273 of SEQ ID NO:5.

Figure 34 depicts a BLAST alignment of human 18431 with a consensus amino acid sequence derived from a ProDomain "SCY1 cDNA FIS kinase weakly serine/threonine-protein similar p23A10.10 for CG12524" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 273 to 305 of the 340 amino acid consensus sequence (SEQ ID NO:36), while the upper amino acid sequence corresponds to the "SCY1 cDNA FIS kinase weakly serine/threonine-

protein similar p23A10.10 for CG12524" domain of human 18431, amino acid residues 744 to 773 of SEQ ID NO:5.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

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Detailed Description

Human 32374

The human 32374 sequence (Figure 1A-B; SEQ ID NO:1), which is approximately 2893 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1041 nucleotides (nucleotides 274-1314 of SEQ ID NO:1; SEQ ID NO:3), including the terminal codon. The coding sequence encodes a 346 amino acid protein (SEQ ID NO:2).

This mature protein form is approximately 346 amino acid residues in length (from about amino acid 1 to amino acid 346 of SEQ ID NO:2). Human 32374 contains the following regions or other structural features:

- one protein kinase domain (PFAM Accession Number PF00069) located at about amino acid residues 1-231 of SEQ ID NO:2;
- one N-glycosylation site (PS00001) located at about amino acids 7-10 of SEQ ID NO:2;
- one Glycosaminoglycan attachment site (PS00002) located at about amino acids 281-284 of SEQ ID NO:2;
- three cAMP- and cGMP-dependent protein kinase phosphorylation sites (PS00004) located at about amino acids 128-131, 204-207 and 245-248 of SEQ ID NO:2;
- three protein kinase C phosphorylation sites (PS00005) located at about amino acids 72-74, 120-122 and 248-250 of SEQ ID NO:2;
- four casein kinase II phosphorylation sites (PS00006) located at about amino acids 137-140, 154-157, 179-182 and 340-343 of SEQ ID NO:2;
- one serine/threonine protein kinases active-site signature (PS00108) located at about amino acids 92-104 of SEQ ID NO:2.

In one embodiment, a 32374 family member can include at least one protein kinase domain (PFAM Accession Number PF00069). Furthermore, a 32374 family member can include at least one N-glycosylation site (PS00001); at least one Glycosaminoglycan

attachment site (PS00002); at least one, two and preferably three cAMP- and cGMP-dependent protein kinase phosphorylation sites (PS00004); at least one, two and preferably three protein kinase C phosphorylation sites (PS00005); at least one, two, three and preferably four casein kinase II phosphorylation sites (PS00006); and at least one
5 serine/threonine protein kinases active-site signature (PS00108).

An additional method to identify the presence of a "protein kinase" domain in a 32374 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a SMART database (Simple Modular Architecture Research Tool,
10 <http://smart.embl-heidelberg.de/>) of HMMs as described in Schultz *et al.* (1998), *Proc. Natl. Acad. Sci. USA* 95:5857 and Schultz *et al.* (2000) *Nucl. Acids Res* 28:231. The database contains domains identified by profiling with the hidden Markov models of the HMMer2 search program (R. Durbin *et al.* (1998) *Biological sequence analysis: probabilistic models of proteins and nucleic acids*. Cambridge University Press.;
15 <http://hmmer.wustl.edu/>). The database also is extensively annotated and monitored by experts to enhance accuracy. A search was performed against the HMM database resulting in the identification of a "serkin_6" domain in the amino acid sequence of human 32374 at about residues 1 to 240 of SEQ ID NO:2 (see Figure 1).

A 32374 polypeptide can include at least one, preferably two "transmembrane
20 domains" or regions homologous with "transmembrane domains". As used herein, the term "transmembrane domain" includes an amino acid sequence of about 10 to 40 amino acid residues in length and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, *e.g.*, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or
25 tryptophans. Transmembrane domains typically have alpha-helical structures and are described in, for example, Zagotta, W.N. *et al.*, (1996) *Annual Rev. Neurosci.* 19:235-263, the contents of which are incorporated herein by reference.

In a preferred embodiment, a 32374 polypeptide or protein has at least one, preferably two transmembrane domains or regions which include at least about 12 to 35
30 more preferably about 14 to 30 or 15 to 25 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "transmembrane domain," *e.g.*, the transmembrane domains of 32374 (*e.g.*, amino acid residues 158-175 and 291-311 of SEQ

ID NO:2). The transmembrane domain of human 32374 is visualized in the hydropathy plot in Figure 2 as regions of about 15 to 25 amino acids where the hydropathy trace is mostly above the horizontal line.

To identify the presence of a "transmembrane" domain in a 32374 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be analyzed by a transmembrane prediction method that predicts the secondary structure and topology of integral membrane proteins based on the recognition of topological models (MEMSAT, Jones *et al.*, (1994) *Biochemistry* 33:3038-3049).

A 32374 polypeptide can include at least one, two, preferably three "non-transmembrane regions." As used herein, the term "non-transmembrane region" includes an amino acid sequence not identified as a transmembrane domain. The non-transmembrane regions in 32374 are located at about amino acids 1-157, 176-290, and 312-346 of SEQ ID NO:2.

The non-transmembrane regions of 32374 include at least one, preferably two cytoplasmic regions. When located at the N-terminus, the cytoplasmic region is referred to herein as the "N-terminal cytoplasmic domain." As used herein, an "N-terminal cytoplasmic domain" includes an amino acid sequence having about 1 to 200, preferably about 1 to 175, more preferably about 1 to 160, or even more preferably about 1 to 157 amino acid residues in length and is located inside of a cell or within the cytoplasm of a cell. The C-terminal amino acid residue of an "N-terminal cytoplasmic domain" is adjacent to an N-terminal amino acid residue of a transmembrane domain in a 32374 protein. For example, an N-terminal cytoplasmic domain is located at about amino acid residues 1 to 157 of SEQ ID NO:2.

In a preferred embodiment, a polypeptide or protein has an N-terminal cytoplasmic domain or a region which includes at least about 5, preferably about 1 to 160, and more preferably about 1 to 157 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with an "N-terminal cytoplasmic domain," e.g., the N-terminal cytoplasmic domain of human 32374 (e.g., residues 1 to 157 of SEQ ID NO:2).

In another embodiment, a cytoplasmic region of a 32374 protein can include the C-terminus and can be a "C-terminal cytoplasmic domain," also referred to herein as a "C-terminal cytoplasmic tail." As used herein, a "C-terminal cytoplasmic domain" includes an

amino acid sequence having a length of at least about 5, preferably about 1 to 40, more preferably about 1 to 34 amino acid residues and is located inside of a cell or within the cytoplasm of a cell. The N-terminal amino acid residue of a "C-terminal cytoplasmic domain" is adjacent to a C-terminal amino acid residue of a transmembrane domain in a 32374 protein. For example, a C-terminal cytoplasmic domain is located at about amino acid residues 312 to 346 of SEQ ID NO:2.

In a preferred embodiment, a 32374 polypeptide or protein has a C-terminal cytoplasmic domain or a region which includes at least about 5, preferably about 1 to 40, and more preferably about 1 to 34 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a C-terminal cytoplasmic domain," *e.g.*, the C-terminal cytoplasmic domain of human 32374 (*e.g.*, residues 312 to 346 of SEQ ID NO:2).

In another embodiment, a 32374 protein includes at least one non-cytoplasmic loop. As used herein, a "non-cytoplasmic loop" includes an amino acid sequence located outside of a cell or within an intracellular organelle. Non-cytoplasmic loops include extracellular domains (*i.e.*, outside of the cell) and intracellular domains (*i.e.*, within the cell). When referring to membrane-bound proteins found in intracellular organelles (*e.g.*, mitochondria, endoplasmic reticulum, peroxisomes microsomes, vesicles, endosomes, and lysosomes), non-cytoplasmic loops include those domains of the protein that reside in the lumen of the organelle or the matrix or the intermembrane space. For example, a "non-cytoplasmic loop" can be found at about amino acid residues 176 to 290 of SEQ ID NO:2.

In a preferred embodiment, a 32374 polypeptide or protein has at least one non-cytoplasmic loop or a region which includes at least about 4, preferably about 5 to 120, more preferably about 6 to 114 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "non-cytoplasmic loop," *e.g.*, at least one non-cytoplasmic loop of human 32374 (*e.g.*, residues 176 to 290 of SEQ ID NO:2).

Human 18431

The human 18431 sequence (Figure 13A-D; SEQ ID NO:4), which is approximately 4136 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 2682 nucleotides (nucleotides 551-3232 of SEQ ID

NO:4; SEQ ID NO:6), including the terminal codon. The coding sequence encodes a 893 amino acid protein (SEQ ID NO:5).

This mature protein form is approximately 893 amino acid residues in length (from about amino acid 1 to amino acid 893 of SEQ ID NO:5). Human 18431 contains the following regions or other structural features:

one protein kinase domain (PFAM Accession Number PF00069) located at about amino acids 43 to 273 of SEQ ID NO:5;

one TBC domain (PFAM Accession Number PF00566) located at about amino acids 463 to 673 of SEQ ID NO:5;

one Rhodanese-like domain (PFAM Accession Number PF00581) located at about amino acids 776 to 883 of SEQ ID NO:5;

one N-glycosylation site (PS00001) located at about amino acids 651-654 of SEQ ID NO:5;

one cAMP- and cGMP-dependent protein kinase phosphorylation site (PS00004) located at about amino acids 260-263 of SEQ ID NO:5;

eleven protein kinase C phosphorylation sites (PS00005) located at about amino acids 35-37, 175-177, 210-212, 259-261, 293-295, 501-503, 704-706, 709-711, 760-762, 784-786, and 789-791 of SEQ ID NO:5;

ten casein kinase II phosphorylation sites (PS00006) located at about amino acids 77-80, 245-248, 263-266, 300-303, 321-324, 374-377, 416-419, 520-523, 538-541 and 764-767 of SEQ ID NO:5;

two tyrosine kinase phosphorylation sites (PS00007) located at about amino acids 320-327 and 431-437 of SEQ ID NO:5;

four N-myristoylation sites (PS00008) located at about amino acids 165-170, 473-478, 481-486 and 725-730 of SEQ ID NO:5;

one amidation site (PS00009) located at about amino acids 199-202 of SEQ ID NO:5.

In one embodiment, a 18431 family member can include at least one protein kinase domain (PFAM Accession Number PF00069); at least one TBC domain (PFAM Accession Number PF00566); and at least one Rhodanese-like domain (PFAM Accession Number PF00581). Furthermore, a 18431 family member can include at least one N-glycosylation site (PS00001); at least one cAMP- and cGMP-dependent protein kinase phosphorylation

sites (PS00004); at least one, two, three, four, five, six, seven, eight, nine, ten and preferably eleven protein kinase C phosphorylation sites (PS00005); at least one, two, three, four, five, six, seven, eight, nine and preferably ten casein kinase II phosphorylation sites (PS00006); at least one and preferably two tyrosine kinase phosphorylation sites (PS00007); at least one, two, three and preferably four N-myristoylation sites (PS00008); and at least one amidation site (PS00009).

An additional method to identify the presence of a "protein kinase" or "TBC" domain in a 18431 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a SMART database (Simple Modular Architecture Research Tool, <http://smart.embl-heidelberg.de/>) of HMMs as described in Schultz *et al.* (1998), *Proc. Natl. Acad. Sci. USA* 95:5857 and Schultz *et al.* (2000) *Nucl. Acids Res* 28:231. The database contains domains identified by profiling with the hidden Markov models of the HMMer2 search program (R. Durbin *et al.* (1998) *Biological sequence analysis: probabilistic models of proteins and nucleic acids*. Cambridge University Press.; <http://hmmer.wustl.edu/>). The database also is extensively annotated and monitored by experts to enhance accuracy. A search was performed against the HMM database resulting in the identification of a "serkin_6" domain in the amino acid sequence of human 18431 at about residues 1 to 273 of SEQ ID NO:5. Additionally, the search identified a "tbc_4" domain in the amino acid sequence of human 18431 at about residues 463 to 674 of SEQ ID NO:5 (see Figure 13).

A 18431 polypeptide can include at least one, preferably two "transmembrane domains" or regions homologous with "transmembrane domains". As used herein, the term "transmembrane domain" includes an amino acid sequence of about 10 to 40 amino acid residues in length and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, *e.g.*, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains typically have alpha-helical structures and are described in, for example, Zagotta, W.N. *et al.*, (1996) *Annual Rev. Neurosci.* 19:235-263, the contents of which are incorporated herein by reference.

In a preferred embodiment, a 18431 polypeptide or protein has at least one, preferably two transmembrane domains or regions which include at least about 12 to 35

more preferably about 14 to 30 or 15 to 25 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "transmembrane domain," e.g., the transmembrane domains of 18431 (e.g., amino acid residues 88-104 and 647-663 of SEQ ID NO:5). The transmembrane domain of human 18431 is visualized in the hydropathy plot in Figure 14 as regions of about 15 to 25 amino acids where the hydropathy trace is mostly above the horizontal line.

To identify the presence of a "transmembrane" domain in a 18431 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be analyzed by a transmembrane prediction method that predicts the secondary structure and topology of integral membrane proteins based on the recognition of topological models (MEMSAT, Jones *et al.*, (1994) *Biochemistry* 33:3038-3049).

A 18431 polypeptide can include at least one, two, preferably three "non-transmembrane regions." As used herein, the term "non-transmembrane region" includes an amino acid sequence not identified as a transmembrane domain. The non-transmembrane regions in 18431 are located at about amino acids 1-87, 105-646, and 664-893 of SEQ ID NO:5.

The non-transmembrane regions of 18431 include at least one, preferably two cytoplasmic regions. When located at the N-terminus, the cytoplasmic region is referred to herein as the "N-terminal cytoplasmic domain." As used herein, an "N-terminal cytoplasmic domain" includes an amino acid sequence having about 1 to 200, preferably about 1 to 100, more preferably about 1 to 90, or even more preferably about 1 to 87 amino acid residues in length and is located inside of a cell or within the cytoplasm of a cell. The C-terminal amino acid residue of an "N-terminal cytoplasmic domain" is adjacent to an N-terminal amino acid residue of a transmembrane domain in a 18431 protein. For example, an N-terminal cytoplasmic domain is located at about amino acid residues 1 to 87 of SEQ ID NO:5.

In a preferred embodiment, a polypeptide or protein has an N-terminal cytoplasmic domain or a region which includes at least about 5, preferably about 1 to 90, and more preferably about 1 to 87 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with an "N-terminal cytoplasmic domain," e.g., the N-terminal cytoplasmic domain of human 18431 (e.g., residues 1 to 87 of SEQ ID NO:5).

In another embodiment, a cytoplasmic region of a 18431 protein can include the C-terminus and can be a "C-terminal cytoplasmic domain," also referred to herein as a "C-terminal cytoplasmic tail." As used herein, a "C-terminal cytoplasmic domain" includes an amino acid sequence having a length of at least about 5, preferably about 1 to 250, more preferably about 1 to 229 amino acid residues and is located inside of a cell or within the cytoplasm of a cell. The N-terminal amino acid residue of a "C-terminal cytoplasmic domain" is adjacent to a C-terminal amino acid residue of a transmembrane domain in a 18431 protein. For example, a C-terminal cytoplasmic domain is located at about amino acid residues 664 to 893 of SEQ ID NO:5.

In a preferred embodiment, a 18431 polypeptide or protein has a C-terminal cytoplasmic domain or a region which includes at least about 5, preferably about 1 to 250, and more preferably about 1 to 229 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a C-terminal cytoplasmic domain," e.g., the C-terminal cytoplasmic domain of human 18431 (e.g., residues 664 to 893 of SEQ ID NO:5).

In another embodiment, a 18431 protein includes at least one non-cytoplasmic loop. As used herein, a "non-cytoplasmic loop" includes an amino acid sequence located outside of a cell or within an intracellular organelle. Non-cytoplasmic loops include extracellular domains (i.e., outside of the cell) and intracellular domains (i.e., within the cell). When referring to membrane-bound proteins found in intracellular organelles (e.g., mitochondria, endoplasmic reticulum, peroxisomes microsomes, vesicles, endosomes, and lysosomes), non-cytoplasmic loops include those domains of the protein that reside in the lumen of the organelle or the matrix or the intermembrane space. For example, a "non-cytoplasmic loop" can be found at about amino acid residues 105 to 646 of SEQ ID NO:5.

In a preferred embodiment, a 18431 polypeptide or protein has at least one non-cytoplasmic loop or a region which includes at least about 4, preferably about 5 to 550, more preferably about 6 to 541 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "non-cytoplasmic loop," e.g., at least one non-cytoplasmic loop of human 18431 (e.g., residues 105 to 646 of SEQ ID NO:5).

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

Plasmids containing the nucleotide sequences encoding human 32374 and 18431 were deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Numbers _____. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 32374 and 18431 proteins contain a significant number of structural characteristics in common with members of the protein kinase family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

As used herein, the term "protein kinase" includes a protein or polypeptide which is capable of playing a role in signaling pathways associated with cellular growth. For example, protein kinases are involved in the regulation of signal transmission from cellular receptors, e.g., growth-factor receptors; entry of cells into mitosis; and the regulation of cytoskeleton function, e.g., actin bundling. Thus, the 32374 or 18431 molecules of the present invention may be involved in: 1) the regulation of transmission of signals from cellular receptors, e.g., cell growth factor receptors; 2) the modulation of the entry of cells into mitosis; 3) the modulation of cellular differentiation; 4) the modulation of cell death; and 5) the regulation of cytoskeleton function, e.g., actin bundling.

Inhibition or over stimulation of the activity of protein kinases involved in signaling pathways associated with cellular growth can lead to perturbed cellular growth, which can in turn lead to cellular growth related disorders. As used herein, a "cellular growth related disorder" includes a disorder, disease, or condition characterized by a deregulation, e.g., an upregulation or a downregulation, of cellular growth. Cellular growth deregulation may be

due to a deregulation of cellular proliferation, cell cycle progression, cellular differentiation and/or cellular hypertrophy.

Additionally, 32374 or 18431 may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, anorexia nervosa, cachexia, lipid disorders, and diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, e.g., inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L., (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

As used herein, the term "protein kinase family members domain" includes an amino acid sequence of about 50-350 amino acid residues in length and having a bit score for the alignment of the sequence to the protein kinase family members domain (HMM) of at least 8. Preferably, a protein kinase family members domain includes at least about 100-300 amino acids, more preferably about 125-275 amino acid residues, or about 150-250 amino acids and has a bit score for the alignment of the sequence to the protein kinase family members domain (HMM) of at least 16 or greater. An alignment of the protein kinase family member domain (amino acids 1-231 of SEQ ID NO:2) of human 32374 with a consensus amino acid sequence derived from a hidden Markov model is depicted in Figure 3. An alignment of the protein kinase family member domain (amino acids 43-273 of SEQ ID NO:5) of human 18431 with a consensus amino acid sequence derived from a hidden Markov model is depicted in Figure 15.

In a preferred embodiment a 32374 or 18431 polypeptide or protein has a "protein kinase family member domain" or a region which includes at least about 50-350 more preferably about 100-300 or 150-250 amino acid residues and has at least about 60%, 70%, 80%, 90%, 95%, 99%, or 100% homology with an "protein kinase family members domain," e.g., the protein kinase family members domain of human 32374 (e.g., amino acid residues 1-231 of SEQ ID NO:2) or the protein kinase family members domain of human 18431 (e.g., amino acid residues 43-273 of SEQ ID NO:5).

To identify the presence of a "protein kinase family member" domain in a 32374 or 18431 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched

against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer et al., (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al., (1990) *Meth. Enzymol.* 183:146-159; Gribskov et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh et al., (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz et al., (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

To identify the presence of a "protein kinase" domain in a 32374 or 18431 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of domains, e.g., the ProDom database (Corpet *et al.* (1999), *Nucl. Acids Res.* 27:263-267). The ProDom protein domain database consists of an automatic compilation of homologous domains. Current versions of ProDom are built using recursive PSI-BLAST searches (Altschul SF *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402; Gouzy *et al.* (1999) *Computers and Chemistry* 23:333-340) of the SWISS-PROT 38 and TREMBL protein databases. The database automatically generates a consensus sequence for each domain. A BLAST search was performed against the HMM database resulting in the identification of a "protein kinase" domain in the amino acid sequence of human 32374 or 18431.

The protein kinase domain is homologous to ProDom family PD193106 ("kinase serin/threonine" SEQ ID NO:8 and 9, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the protein kinase domain (amino acids 226-285 and 321-346 of SEQ ID NO:2) of human 32374 with a consensus amino acid sequence (SEQ ID NO:8 and 9) derived from a hidden Markov model is depicted in Figures 4 and 5. The consensus sequence for SEQ ID NO:8 is 95% identical over amino acids 226 to 285 and for SEQ ID NO:9 is 100% identical over amino acids 321 to 346 of SEQ ID NO:2 as shown in Figures 4 and 5.

The protein kinase domain is also homologous to ProDom family PD057870 ("kinase serine/threonine-protein X C01C4.3 chromosome ATP-binding transferase 2.7.1")

SEQ ID NO:10, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>).

An alignment of the protein kinase domain (amino acids 166-245 of SEQ ID NO:2) of human 32374 with a consensus amino acid sequence (SEQ ID NO:10) derived from a hidden Markov model is depicted in Figure 6. The consensus sequence for SEQ ID NO:10 is 30% identical over amino acids 166 to 245 of SEQ ID NO:2 as shown in Figure 6.

The protein kinase domain is also homologous to ProDom family PD156063 ("kinase CP0625 serine/threonine S/T TC0422 serine/threonine-protein" SEQ ID NO:15, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the protein kinase domain (amino acids 30-189 of SEQ ID NO:2) of human 32374 with a consensus amino acid sequence (SEQ ID NO:15) derived from a hidden Markov model is depicted in Figure 11. The consensus sequence for SEQ ID NO:15 is 22% identical over amino acids 30 to 189 of SEQ ID NO:2 as shown in Figure 11.

The protein kinase domain is also homologous to ProDom family PD325057 ("kinase kinase-related serine/threonine serine/threonine-protein" SEQ ID NO:16, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the protein kinase domain (amino acids 29-262 of SEQ ID NO:2) of human 32374 with a consensus amino acid sequence (SEQ ID NO:16) derived from a hidden Markov model is depicted in Figure 12. The consensus sequence for SEQ ID NO:16 is 26% identical over amino acids 29 to 262 of SEQ ID NO:2 as shown in Figure 12.

The protein kinase domain is also homologous to ProDom family PD000001 ("kinase serine/threonine-protein transferase receptor ATP-binding 2..1.-tyrosine-protein phosphorylation precursor" SEQ ID NO:25 and 26, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the protein kinase domain (amino acids 72-140 and 152-276 of SEQ ID NO:5) of human 18431 with a consensus amino acid sequence (SEQ ID NO:25 and 26) derived from a hidden Markov model is depicted in Figures 23 and 24. The consensus sequence for SEQ ID NO:25 is 36% identical over amino acids 72 to 140 and for SEQ ID NO:26 is 23% identical over amino acids 152 to 276 of SEQ ID NO:5 as shown in Figures 25 and 26.

The protein kinase domain is also homologous to ProDom family PD043026 ("SCY1 cDNA FIS kinase weakly serine/threonine-protein similar P23A10.10 for CG12524" SEQ ID NO:35 and 36, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the protein kinase domain

(amino acids 112-273 and 744-773 of SEQ ID NO:5) of human 18431 with a consensus amino acid sequence (SEQ ID NO:35 and 36) derived from a hidden Markov model is depicted in Figures 33 and 34. The consensus sequence for SEQ ID NO:35 is 22% identical over amino acids 112 to 273 and for SEQ ID NO:36 is 33% identical over amino acids 744 to 773 of SEQ ID NO:5 as shown in Figures 35 and 36.

In another embodiment, the isolated proteins of the present invention, preferably 32374 proteins, are identified based on the presence of at least one Ser/Thr kinase site. As used herein, the term "Ser/Thr kinase site" includes an amino acid sequence of about 200-400 amino acid residues in length, preferably 200-300 amino acid residues in length, and more preferably 250-300 amino acid residues in length, which is conserved in kinases which phosphorylate serine and threonine residues and found in the catalytic domain of Ser/Thr kinases. Preferably, the Ser/Thr kinase site includes the following amino acid consensus sequence X₉-g-X-G-X₄-V-X₁₂-K-X-(10-19)-E-X₆₆-h-X₈-h-r-D-X-K-X₂-N-X₁₇-K-X₂-D-f-g-X₂₁-p-X₁₃-w-X₃-g-X₅₅-R-X₁₄-h-X₃ (SEQ ID NO:37) (where invariant residues are indicated by upper case letters and nearly invariant residues are indicated by lower case letters). The nearly invariant residues are usually found in most Ser/Thr kinase sites, but can be replaced by other amino acids which, preferably, have similar characteristics. For example, a nearly invariant hydrophobic amino acid in the above amino acid consensus sequence would most likely be replaced by another hydrophobic amino acid. Ser/Thr kinase domains are described in, for example, Levin D.E. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8272-76, the contents of which are incorporated herein by reference. Amino acid residues 1-231 of the 32374 protein comprise a Ser/Thr kinase domain. Amino acid residues 43-273 of the 18431 protein comprise a Ser/Thr kinase domain.

Accordingly, another embodiment of the invention features isolated 32374 proteins and polypeptides having a 32374 activity. Preferred proteins are 32374 proteins having at least one Ser/Thr kinase. Additional preferred proteins have at least one Ser/Thr kinase site and preferably a 32374 activity. Additional preferred proteins have at least one Ser/Thr kinase site and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3.

Accordingly, another embodiment of the invention features isolated 18431 proteins and polypeptides having a 18431 activity. Preferred proteins are 18431 proteins having at least one Ser/Thr kinase. Additional preferred proteins have at least one Ser/Thr kinase site and preferably a 18431 activity. Additional preferred proteins have at least one Ser/Thr kinase site and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:6.

The 32374 nucleic acids encodes a polypeptide with similarities to known Ser/Thr kinases. Thus the 32374 encoded polypeptide is expected to be a kinase and function in the phosphorylation of protein substrates. Additionally, the 32374 nucleic acids can be used in known or novel screens and assays for kinase encoding nucleic acids to distinguish it from other distinct nucleic acids. Alternatively, the nucleic acid sequences can be used in the preparation of phylogenetic trees and relationships between organisms.

The 18431 nucleic acids encodes a polypeptide with similarities to known Ser/Thr kinases. Thus the 18431 encoded polypeptide is expected to be a kinase and function in the phosphorylation of protein substrates. Additionally, the 18431 nucleic acids can be used in known or novel screens and assays for kinase encoding nucleic acids to distinguish it from other distinct nucleic acids. Alternatively, the nucleic acid sequences can be used in the preparation of phylogenetic trees and relationships between organisms.

As used herein, a "32374 or 18431 activity", "biological activity of 32374 or 18431" or "functional activity of 32374 or 18431", refers to an activity exerted by a 32374 or 18431 protein, polypeptide or nucleic acid molecule on e.g., a 32374- or 18431-responsive cell or on a 32374 or 18431 substrate, e.g., a lipid or protein substrate, as determined *in vivo* or *in vitro*.

As the 32374 or 18431 polypeptides of the invention may modulate 32374- or 18431-mediated activities, they may be useful for developing novel diagnostic and therapeutic agents for 32374- or 18431-mediated or related disorders, as described below.

Accordingly, 32374 or 18431 protein may mediate various disorders, including cellular proliferative and/or differentiative disorders, pain or metabolic disorders, and brain disorders.

Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g.,

leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

The terms "cancer" or "neoplasms" include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genitourinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

The 32374 or 18431 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of proliferative disorders. E.g., such disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic

disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof.

Preferably, the diseases arise from poorly differentiated acute leukemias, e.g.,

erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary

5 myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML),

acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L., (1991) *Crit. Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies

include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-

lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic

10 leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM).

Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin

lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell

leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular

lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

15 Disorders involving the brain include, but are not limited to, disorders involving

neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal

cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and

hydrocephalus; malformations and developmental diseases, such as neural tube defects,

forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia;

20 perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia,

and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral

ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply,

intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage,

subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations,

25 hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and

hypertensive encephalopathy; infections, such as acute meningitis, including acute

pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative

infections, including brain abscess, subdural empyema, and extradural abscess, chronic

bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis,

30 and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne

(Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2,

Varicella-zoster virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human

immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible
5 spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem,
10 including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor
15 neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial
20 encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B₁) deficiency and vitamin B₁₂ deficiency, neurologic sequelae of metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury;
25 tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and
30 pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (malignant schwannoma), and neurocutaneous syndromes

(phakomatoses), including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

The 32374 or 18431 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 or SEQ ID NO:5 are collectively referred to as

5 "polypeptides or proteins of the invention" or "32374 or 18431 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "32374 or 18431 nucleic acids." 32374 or 18431 molecules refer to 32374 or 18431 nucleic acids, polypeptides, and antibodies.

10 As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

15 The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the
20 nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or
25 culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

30 As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one

or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, or SEQ ID NO:4, corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 32374 or 18431 protein, preferably a mammalian 32374 or 18431 protein, and can further include non-coding regulatory sequences, and introns.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of 32374 or 18431 protein having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-32374 or -18431 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-32374 or -18431 chemicals. When the 32374 or 18431 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably

less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 32374 or 18431 (e.g., the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the protein kinase family members domain, are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 32374 or 18431 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 32374 or 18431 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 32374 or 18431 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a 32374 or 18431 protein includes a fragment of a 32374 or 18431 protein which participates in an interaction between a 32374 or 18431 molecule and a non-32374 or -18431 molecule. Biologically active portions of a 32374 or 18431 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 32374 or 18431

protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5, which include less amino acids than the full length 32374 or 18431 proteins, and exhibit at least one activity of a 32374 or 18431 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 32374 or 18431 protein, e.g., a protein kinase family member activity. A biologically active portion of a 32374 or 18431 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 32374 or 18431 protein can be used as targets for developing agents which modulate a 32374 or 18431 mediated activity, e.g., a protein kinase family member activity.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 32374 amino acid sequence of SEQ ID NO:2 having 346 amino acid residues, at least 104, preferably at least 138, more preferably at least 173, even more preferably at least 208, and even more preferably at least 242, 277, 311 or 346 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred

embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al., (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 32374 or 18431 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 32374 or 18431 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild

type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

Isolated Nucleic Acid Molecules

In one aspect, the invention provides, isolated or purified, nucleic acid molecules that encode a 32374 or 18431 polypeptide described herein, e.g., a full length 32374 or 18431 protein or a fragment thereof, e.g., a biologically active portion of 32374 or 18431 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify nucleic acid molecule encoding a polypeptide of the invention, 32374 or 18431 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:4, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid

molecule includes sequences encoding the human 32374 or 18431 protein (i.e., "the coding region", from nucleotides 274-1314 of SEQ ID NO:1, or from nucleotides 551-3232 of SEQ ID NO:4 including the terminal codon), as well as 5' untranslated sequences (nucleotides 1-273 of SEQ ID NO:1, or nucleotides 1-550 of SEQ ID NO:4).

5 Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 or SEQ ID NO:4 (e.g., nucleotides 274-1314 of SEQ ID NO:1, corresponding to SEQ ID NO:3, or nucleotides 551-3232 of SEQ ID NO:4, corresponding to SEQ ID NO:6) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature
10 protein of SEQ ID NO:2 or SEQ ID NO:5.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion
15 of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID
20 NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the
25 nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, e.g., SEQ ID NO:1, or SEQ ID NO:4, the comparison is made with the full length of the reference sequence. Where the
30 isolated nucleic acid molecule is shorter than the reference sequence, e.g., shorter than SEQ ID NO:1, or SEQ ID NO:4, the comparison is made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

32374 or 18431 Nucleic Acid Fragments

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a 32374 or 18431 protein, e.g., an immunogenic or biologically active portion of a 32374 or 18431 protein. A fragment can comprise: nucleotides 274-966 of SEQ ID NO:1, or nucleotides 677-1369 of SEQ ID NO:4, which encodes a protein kinase family members domain of human 32374 or 18431, respectively. The nucleotide sequence determined from the cloning of the 32374 or 18431 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 32374 or 18431 family members, or fragments thereof, as well as 32374 or 18431 homologues, or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region. Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 150 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not be construed as encompassing those fragments that may have been disclosed prior to the invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or more domain, region, or functional site described herein. Thus, for example, the nucleic acid fragment can include a protein kinase family members domain. In a preferred embodiment the fragment is at least, 50, 100, 200, 300, 400, 500, 600, 700, or 900 base pairs in length.

32374 or 18431 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75

consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the
5 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein.
10 If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes a protein kinase family members domain (e.g., about amino acid
15 residues 1-231 of SEQ ID NO:2 or 43-273 SEQ ID NO:5).

In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a 32374 or 18431 sequence, e.g., a region described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or
20 differs by one base from a sequence disclosed herein or from a naturally occurring variant. E.g., primers suitable for amplifying all or a portion of any of the following regions are provided: a protein kinase family members domain (e.g., about amino acid residues 1-231 of SEQ ID NO:2 or 43-273 of SEQ ID NO:5).

A nucleic acid fragment can encode an epitope bearing region of a polypeptide
25 described herein.

A nucleic acid fragment encoding a "biologically active portion of a 32374 or 18431 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, which
30 encodes a polypeptide having a 32374 or 18431 biological activity (e.g., the biological activities of the 32374 or 18431 proteins as described herein), expressing the encoded portion of the 32374 or 18431 protein (e.g., by recombinant expression *in vitro*) and

assessing the activity of the encoded portion of the 32374 or 18431 protein. For example, a nucleic acid fragment encoding a biologically active portion of 32374 or 18431 includes a protein kinase family members domain (e.g., about amino acid residues 1-231 of SEQ ID NO:2 or 43-273 of SEQ ID NO:5). A nucleic acid fragment encoding a biologically active
5 portion of a 32374 or 18431 polypeptide, may comprise a nucleotide sequence which is greater than 300-1200 or more nucleotides in length.

In preferred embodiments, nucleic acids include a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of
10 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ____.

32374 or 18431 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 32374 or 18431 proteins as those
20 encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2 or SEQ ID NO:5. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped"
25 out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the inventor can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in E. coli, yeast, human, insect, or CHO
30 cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non-naturally

occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 or a fragment of this sequence. Such nucleic acid molecules can readily be obtained as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:3 or SEQ ID NO:6, or a fragment of this sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 32374 or 18431 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 32374 or 18431 gene. Preferred variants include those that are correlated with protein kinase family members activity.

Allelic variants of 32374 or 18431, e.g., human 32374 or 18431, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 32374 or 18431 protein within a population that maintain the ability to modulate the phosphorylation state of itself or another protein or polypeptide. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or SEQ ID NO:5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 32374 or 18431, e.g., human 32374 or 18431, protein within a population that do not have

the ability to attach an acyl chain to a lipid precursor. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

5 Moreover, nucleic acid molecules encoding other 32374 or 18431 family members and, thus, which have a nucleotide sequence which differs from the 32374 or 18431 sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention.

10 Antisense Nucleic Acid Molecules, Ribozymes and Modified 32374 or 18431 Nucleic Acid Molecules

 In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 32374 or 18431. An "antisense" nucleic acid can include a nucleotide
15 sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 32374 or 18431 coding strand, or to only a portion thereof (e.g., the coding region of human 32374 or 18431 corresponding to SEQ ID NO:3 or SEQ ID NO:6). In
20 another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 32374 or 18431 (e.g., the 5' and 3' untranslated regions).

 An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 32374 or 18431 mRNA, but more preferably is an oligonucleotide
25 which is antisense to only a portion of the coding or noncoding region of 32374 or 18431 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 32374 or 18431 mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65,
30 70, 75, 80, or more nucleotides in length.

 An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example,

an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 32374 or 18431 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al., (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a 32374- or 18431-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 32374 or

18431 cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach, (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 32374- or 18431-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, 32374 or 18431 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

32374 or 18431 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 32374 or 18431 (e.g., the 32374 or 18431 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 32374 or 18431 gene in target cells. See generally, Helene, C., (1991) *Anticancer Drug Des.* 6(6):569-84; Helenc, C. et al., (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J., (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

A 32374 or 18431 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al., (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for

specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al., (1996) *supra*; Perry-O'Keefe et al., *Proc. Natl. Acad. Sci.* 93: 14670-675.

5 PNAs of 32374 or 18431 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 32374 or 18431 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-
10 directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B., (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al., (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport
15 across the cell membrane (see, e.g., Letsinger et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al., (1988) *Bio-Techniques* 6:958-976) or intercalating
20 agents. (See, e.g., Zon, (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 32374 or 18431 nucleic
25 acid of the invention, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantitating the presence of the 32374 or 18431 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

Isolated 32374 or 18431 Polypeptides

In another aspect, the invention features, an isolated 32374 or 18431 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-32374 or -18431 antibodies. 32374 or 18431 protein can be isolated from cells or tissue sources using standard protein purification techniques. 32374 or 18431 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same postranslational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of postranslational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

In a preferred embodiment, a 32374 or 18431 polypeptide has one or more of the following characteristics:

(i) it has the ability to reversibly phosphorylate proteins in order to regulate protein activity in eukaryotic cells;

(ii) it has a molecular weight, e.g., a deduced molecular weight, amino acid composition or other physical characteristic of the polypeptide of SEQ ID NO:2 or SEQ ID NO:5;

(iii) it has an overall sequence similarity of at least 50%, preferably at least 60%, more preferably at least 70, 80, 90, or 95%, with a polypeptide of SEQ ID NO:2 or SEQ ID NO:5;

(iv) it has a protein kinase family members domain which preferably has an overall sequence similarity of about 70%, 80%, 90% or 95% with amino acid residues 1-231 of SEQ ID NO:2 or 43-273 of SEQ ID NO:5;

(v) it has at least 70%, preferably 80%, and most preferably 95% of the cysteines found in the amino acid sequence of the native protein.

In a preferred embodiment the 32374 or 18431 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5 by at least one residue

but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non-essential residue or a conservative substitution. In a preferred embodiment the differences are not in the protein kinase family members domain. In another preferred embodiment one or more differences are in non-active site residues, e.g. outside of the protein kinase family members domain.

Other embodiments include a protein that contain one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity. Such 32374 or 18431 proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID NO:5, yet retain biological activity.

In one embodiment, a biologically active portion of a 32374 or 18431 protein includes a protein kinase family members domain. In another embodiment, a biologically active portion of a 18431 protein includes a TBC domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 32374 or 18431 protein.

In a preferred embodiment, the 32374 or 18431 protein has an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5. In other embodiments, the 32374 or 18431 protein is substantially identical to SEQ ID NO:2 or SEQ ID NO:5. In yet another embodiment, the 32374 or 18431 protein is substantially identical to SEQ ID NO:2 or SEQ ID NO:5 and retains the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:5, as described in detail above. Accordingly, in another embodiment, the 32374 or 18431 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:2 or SEQ ID NO:5.

32374 or 18431 Chimeric or Fusion Proteins

In another aspect, the invention provides 32374 or 18431 chimeric or fusion proteins. As used herein, a 32374 or 18431 "chimeric protein" or "fusion protein" includes a 32374 or 18431 polypeptide linked to a non-32374 or -18431 polypeptide. A "non-32374

or -18431 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 32374 or 18431 protein, e.g., a protein which is different from the 32374 or 18431 protein and which is derived from the same or a different organism. The 32374 or 18431 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a 32374 or 18431 amino acid sequence. In a preferred embodiment, a 32374 or 18431 fusion protein includes at least one (or two) biologically active portion of a 32374 or 18431 protein. The non-32374 or -18431 polypeptide can be fused to the N-terminus or C-terminus of the 32374 or 18431 polypeptide.

The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-32374 or -18431 fusion protein in which the 32374 or 18431 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 32374 or 18431. Alternatively, the fusion protein can be a 32374 or 18431 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of 32374 or 18431 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

The 32374 or 18431 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 32374 or 18431 fusion proteins can be used to affect the bioavailability of a 32374 or 18431 substrate. 32374 or 18431 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 32374 or 18431 protein; (ii) mis-regulation of the 32374 or 18431 gene; and (iii) aberrant post-translational modification of a 32374 or 18431 protein.

Moreover, the 32374- or 18431-fusion proteins of the invention can be used as immunogens to produce anti-32374 or -18431 antibodies in a subject, to purify 32374 or 18431 ligands and in screening assays to identify molecules which inhibit the interaction of 32374 or 18431 with a 32374 or 18431 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A 32374- or 18431-encoding nucleic acid can be cloned into

such an expression vector such that the fusion moiety is linked in-frame to the 32374 or 18431 protein.

Variants of 32374 or 18431 Proteins

5 In another aspect, the invention also features a variant of a 32374 or 18431 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 32374 or 18431 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a 32374 or 18431 protein. An agonist of the 32374 or 18431 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 32374 or 18431 protein. An antagonist of a 32374 or 18431 protein can inhibit one or more of the activities of the naturally occurring form of the 32374 or 18431 protein by, for example, competitively modulating a 32374- or 18431-mediated activity of a 32374 or 18431 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 32374 or 18431 protein.

Variants of a 32374 or 18431 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 32374 or 18431 protein for agonist or antagonist activity.

Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of a 32374 or 18431 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 32374 or 18431 protein.

Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 32374 or 18431 variants (Arkin and Yourvan, (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al., (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated 32374 or 18431 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to 32374 or 18431 in a substrate-dependent manner. The transfected cells are then contacted with 32374 or 18431 and the effect of the expression of the mutant on signaling by the 32374 or 18431 substrate can be detected, e.g., by measuring protein kinase family members activity. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the 32374 or 18431 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a 32374 or 18431 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring 32374 or 18431 polypeptide, e.g., a naturally occurring 32374 or 18431 polypeptide. The method includes: altering the sequence of a 32374 or 18431 polypeptide, e.g., altering the sequence, e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a 32374 or 18431 polypeptide a biological activity of a naturally occurring 32374 or 18431 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a 32374 or 18431 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

Anti-32374 or -18431 Antibodies

In another aspect, the invention provides an anti-32374 or -18431 antibody. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin.

The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human, non-human, e.g., murine, or single chain antibody. In a preferred

embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

A full-length 32374 or 18431 protein or, antigenic peptide fragment of 32374 or 18431 can be used as an immunogen or can be used to identify anti-32374 or -18431 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 32374 or 18431 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 and encompasses an epitope of 32374 or 18431. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 32374 or 18431 which include, e.g., residues 236-266 of SEQ ID NO:2 or 441-466 of SEQ ID NO:5 can be, e.g., used as immunogens, or used to characterize the specificity of an antibody or antibodies against what are believed to be hydrophilic regions of the 32374 or 18431 protein. Similarly, a fragment of 32374 or 18431 which includes, e.g., residues 291-311 of SEQ ID NO:2 or 471-491 of SEQ ID NO:5 can be used to make an antibody against what is believed to be a hydrophobic region of the 32374 or 18431 protein; a fragment of 32374 or 18431 which includes residues 1-231 of SEQ ID NO:2 or 43-273 of SEQ ID NO:5 can be used to make an antibody against the protein kinase family members region of the 32374 or 18431 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

In a preferred embodiment the antibody fails to bind an Fc receptor, e.g. it is a type which does not support Fc receptor binding or has been modified, e.g., by deletion or other mutation, such that it does not have a functional Fc receptor binding region.

Preferred epitopes encompassed by the antigenic peptide are regions of 32374 or 18431 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 32374 or 18431 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 32374 or 18431 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody binds an epitope on any domain or region on 32374 or 18431 proteins described herein.

Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, e.g., therapeutic treatment (and some diagnostic applications) of human patients.

The anti-32374 or -18431 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. et al., *Ann. NY Acad. Sci.* 1999 Jun 30;880:263-80; and Reiter, Y., *Clin. Cancer Res.* 1996 Feb;2(2):245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 32374 or 18431 protein.

An anti-32374 or -18431 antibody (e.g., monoclonal antibody) can be used to isolate 32374 or 18431 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-32374 or -18431 antibody can be used to detect 32374 or 18431 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-32374 or -18431 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the

term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

5 A vector can include a 32374 or 18431 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include
10 those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion
15 proteins or polypeptides, encoded by nucleic acids as described herein (e.g., 32374 or 18431 proteins, mutant forms of 32374 or 18431 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 32374 or 18431 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression
20 vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7
polymerase.

25 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2)
30 to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein

to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S., (1988) *Gene* 67:31-40),
5 pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 32374 or 18431 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific
10 for 32374 or 18431 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in
15 host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992)
20 *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 32374 or 18431 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

25 When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples
30 of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al., (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton,

(1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore, (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al., (1983) *Cell* 33:729-740; Queen and Baltimore, (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al., (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss, (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman, (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 32374 or 18431 nucleic acid molecule within a recombinant expression vector or a 32374 or 18431 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 32374 or 18431 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or

mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation

A host cell of the invention can be used to produce (i.e., express) a 32374 or 18431 protein. Accordingly, the invention further provides methods for producing a 32374 or 18431 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 32374 or 18431 protein has been introduced) in a suitable medium such that a 32374 or 18431 protein is produced. In another embodiment, the method further includes isolating a 32374 or 18431 protein from the medium or the host cell.

In another aspect, the invention features, a cell or purified preparation of cells which include a 32374 or 18431 transgene, or which otherwise misexpress 32374 or 18431. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a 32374 or 18431 transgene, e.g., a heterologous form of a 32374 or 18431, e.g., a gene derived from humans (in the case of a non-human cell). The 32374 or 18431 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous 32374 or 18431, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed 32374 or 18431 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject 32374 or 18431 polypeptide.

Also provided are cells or a purified preparation thereof, e.g., human cells, in which an endogenous 32374 or 18431 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 32374 or 18431 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the

cell such that the inserted regulatory element is operably linked to the endogenous 32374 or 18431 gene. For example, an endogenous 32374 or 18431 gene, e.g., a gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 32374 or 18431 protein and for identifying and/or evaluating modulators of 32374 or 18431 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 32374 or 18431 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 32374 or 18431 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 32374 or 18431 transgene in its genome and/or expression of 32374 or 18431 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic

animals carrying a transgene encoding a 32374 or 18431 protein can further be bred to other transgenic animals carrying other transgenes.

32374 or 18431 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed herein.

Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express a 32374 or 18431 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a 32374 or 18431 mRNA (e.g., in a biological sample) or a genetic alteration in a 32374 or 18431 gene, and to modulate 32374 or 18431 activity, as described further below. The 32374 or 18431 proteins can be used to treat disorders characterized by insufficient or excessive production of a 32374 or 18431 substrate or production of 32374 or 18431 inhibitors. In addition, the 32374 or 18431 proteins can be used to screen for naturally occurring 32374 or 18431 substrates, to screen for drugs or compounds which modulate 32374 or 18431 activity, as well as to treat disorders characterized by insufficient or excessive production of 32374 or 18431 protein or production of 32374 or 18431 protein forms which have decreased, aberrant or unwanted activity compared to 32374 or 18431 wild-type protein. Such disorders include those characterized by aberrant signaling or aberrant, e.g., hyperproliferative, cell growth. Moreover, the anti-32374 or -18431 antibodies of the invention can be used to detect and isolate 32374 or 18431 proteins, regulate the bioavailability of 32374 or 18431 proteins, and modulate 32374 or 18431 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject 32374 or 18431 polypeptide is provided. The method includes: contacting the compound with the subject 32374 or 18431 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject 32374 or 18431 polypeptide. This method can be performed in vitro, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject 32374 or 18431 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 32374 or 18431 polypeptide. Screening methods are discussed in more detail below.

Screening Assays:

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to 32374 or 18431 proteins, have a stimulatory or inhibitory effect on, for example, 32374 or 18431 expression or 32374 or 18431 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 32374 or 18431 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 32374 or 18431 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 32374 or 18431 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 32374 or 18431 protein or polypeptide or a biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R.N. et al., *J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound'

library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

5 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.*, (1994). *J. Med. Chem.* 37:2678; Cho *et al.*, (1993) *Science* 261:1303; Carrell *et al.*, (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.*, (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.*, (1994) *J. Med. Chem.* 37:1233.

10 Libraries of compounds may be presented in solution (e.g., Houghten, (1992) *Biotechniques* 13:412-421), or on beads (Lam, (1991) *Nature* 354:82-84), chips (Fodor, (1993) *Nature* 364:555-556), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or on phage (Scott and Smith, (1990) *Science* 249:386-390); (Devlin, (1990) *Science* 249:404-406); (Cwirla *et al.*, (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici, (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

15 In one embodiment, an assay is a cell-based assay in which a cell which expresses a 32374 or 18431 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate 32374 or 18431 activity is determined. Determining the ability of the test compound to modulate 32374 or 18431 activity can be accomplished by monitoring, for example, protein kinase family members activity. The cell, for example, can be of mammalian origin, e.g., human. Cell homogenates, or fractions, preferably membrane containing fractions, can also be tested.

25 The ability of the test compound to modulate 32374 or 18431 binding to a compound, e.g., a 32374 or 18431 substrate, or to bind to 32374 or 18431 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to 32374 or 18431 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, 32374 or 18431 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 32374 or 18431 binding to a 32374 or 18431 substrate in a complex. For example,

compounds (e.g., 32374 or 18431 substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (e.g., a 32374 or 18431 substrate) to interact with 32374 or 18431 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 32374 or 18431 without the labeling of either the compound or the 32374 or 18431. McConnell, H. M. et al., (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 32374 or 18431.

In yet another embodiment, a cell-free assay is provided in which a 32374 or 18431 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 32374 or 18431 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 32374 or 18431 proteins to be used in assays of the present invention include fragments which participate in interactions with non-32374 or -18431 molecules, e.g., fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (e.g., 32374 or 18431 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-

propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

In one embodiment, assays are performed where the ability of an agent to block protein kinase family members activity within a cell is evaluated.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of the 32374 or 18431 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C., (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al., (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase

can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 32374 or 18431, an anti-32374 or -18431 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 32374 or 18431 protein, or interaction of a 32374 or 18431 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/32374 or 18431 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 32374 or 18431 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 32374 or 18431 binding or activity determined using standard techniques.

Other techniques for immobilizing either a 32374 or 18431 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 32374 or 18431 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of

complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 32374 or 18431 protein or target molecules but which do not interfere with binding of the 32374 or 18431 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 32374 or 18431 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 32374 or 18431 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 32374 or 18431 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci* 1993 Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H., *J Mol. Recognit.* 1998 Winter;11(1-6):141-8; Hage, D.S., and Tweed, S.A., *J. Chromatogr. B Biomed. Sci. Appl.* 1997 Oct 10;699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the 32374 or 18431 protein or biologically active portion thereof with a known compound which binds 32374 or 18431 to form an assay mixture, contacting the assay mixture with a test compound, and

determining the ability of the test compound to interact with a 32374 or 18431 protein, wherein determining the ability of the test compound to interact with a 32374 or 18431 protein includes determining the ability of the test compound to preferentially bind to 32374 or 18431 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 32374 or 18431 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 32374 or 18431 protein through modulation of the activity of a downstream effector of a 32374 or 18431 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), e.g., a substrate, a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and

mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format.

5 Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere
10 with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after
15 complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments.

20 Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will
25 remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or
30 indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 32374 or 18431 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al., (1993) *Cell* 72:223-232; Madura et al., (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al., (1993) *Biotechniques* 14:920-924; Iwabuchi et al., (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 32374 or 18431 ("32374- or 18431-binding proteins" or "32374- or 18431-bp") and are involved in 32374 or 18431 activity. Such 32374- or 18431-bps can be activators or inhibitors of signals by the 32374 or 18431 proteins or 32374 or 18431 targets as, for example, downstream elements of a 32374- or 18431-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 32374 or 18431 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively the:

32374 or 18431 protein can be fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 32374- or 18431-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 32374 or 18431 protein.

In another embodiment, modulators of 32374 or 18431 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of 32374 or 18431 mRNA or protein evaluated relative to the level of expression of 32374 or 18431 mRNA or protein in the absence of the candidate compound. When expression of 32374 or 18431 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 32374 or 18431 mRNA or protein expression. Alternatively, when expression of 32374 or 18431 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 32374 or 18431 mRNA or protein expression. The level of 32374 or 18431 mRNA or protein expression can be determined by methods described herein for detecting 32374 or 18431 mRNA or protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 32374 or 18431 protein can be confirmed *in vivo*, e.g., in an animal.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a 32374 or 18431 modulating agent, an antisense 32374 or 18431 nucleic acid molecule, a 32374- or 18431-specific antibody, or a 32374- or 18431-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their
5 respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 32374 or 18431 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

10 The 32374 or 18431 nucleotide sequences or portions thereof can be used to map the location of the 32374 or 18431 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 32374 or 18431 sequences with genes associated with disease.

15 Briefly, 32374 or 18431 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 32374 or 18431 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 32374 or 18431 sequences will yield an amplified fragment.

20 A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al., (1983) *Science* 220:919-924).

25 Other mapping strategies e.g., in situ hybridization (described in Fan, Y. et al., (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 32374 or 18431 to a chromosomal location.

30 Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably

1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single
5 chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

10 Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can
15 then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al., (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 32374 or 18431 gene, can be determined. If a
20 mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to
25 confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

32374 or 18431 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique,
30 an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification.

The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome.

5 Thus, the 32374 or 18431 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to
10 allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in
15 the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or SEQ ID NO:4 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or SEQ ID NO:6 are used, a more appropriate number of primers for positive
20 individual identification would be 500-2,000.

If a panel of reagents from 32374 or 18431 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from
25 extremely small tissue samples.

Use of Partial 32374 or 18431 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from
30 very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The 32374 or 18431 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., a tissue containing protein kinase family members activity. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 32374 or 18431 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., 32374 or 18431 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 32374 or 18431.

Such disorders include, e.g., a disorder associated with the misexpression of 32374 or 18431, or lipid metabolism related disorder.

The method includes one or more of the following:
detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 32374 or 18431 gene, or detecting the presence or absence of a

mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 32374 or 18431 gene;

5 detecting, in a tissue of the subject, the misexpression of the 32374 or 18431 gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA ;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of a 32374 or 18431 polypeptide.

10 In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 32374 or 18431 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1 or SEQ ID NO:4 naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the 32374 or 18431 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

20 In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 32374 or 18431 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 32374 or 18431.

25 Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of a 32374 or 18431 gene, an abnormal structure being indicative of risk for the disorder.

30 In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 32374 or 18431 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

Diagnostic and Prognostic Assays

The presence, level, or absence of 32374 or 18431 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 32374 or 18431 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 32374 or 18431 protein such that the presence of 32374 or 18431 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 32374 or 18431 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 32374 or 18431 genes; measuring the amount of protein encoded by the 32374 or 18431 genes; or measuring the activity of the protein encoded by the 32374 or 18431 genes.

The level of mRNA corresponding to the 32374 or 18431 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 32374 or 18431 nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:4, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 32374 or 18431 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array. A skilled artisan can adapt

known mRNA detection methods for use in detecting the level of mRNA encoded by the 32374 or 18431 genes.

The level of mRNA in a sample that is encoded by one of 32374 or 18431 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, 1991, *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 32374 or 18431 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 32374 or 18431 mRNA, or genomic DNA, and comparing the presence of 32374 or 18431 mRNA or genomic DNA in the control sample with the presence of 32374 or 18431 mRNA or genomic DNA in the test sample.

A variety of methods can be used to determine the level of protein encoded by 32374 or 18431. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling

(i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect 32374 or 18431 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 32374 or 18431 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 32374 or 18431 protein include introducing into a subject a labeled anti-32374 or -18431 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 32374 or 18431 protein, and comparing the presence of 32374 or 18431 protein in the control sample with the presence of 32374 or 18431 protein in the test sample.

The invention also includes kits for detecting the presence of 32374 or 18431 in a biological sample. For example, the kit can include a compound or agent capable of detecting 32374 or 18431 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 32374 or 18431 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein-stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can

be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 32374 or 18431 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 32374 or 18431 expression or activity is identified. A test sample is obtained from a subject and 32374 or 18431 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 32374 or 18431 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 32374 or 18431 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant/or unwanted 32374 or 18431 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a cellular growth related disorder.

The methods of the invention can also be used to detect genetic alterations in a 32374 or 18431 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 32374 or 18431 protein activity or nucleic acid expression, such as a cellular growth related disorder. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 32374- or 18431-protein, or the mis-expression of the 32374 or 18431 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 32374 or 18431 gene; 2) an addition of one or more nucleotides to a 32374 or 18431 gene; 3) a substitution of one or

more nucleotides of a 32374 or 18431 gene, 4) a chromosomal rearrangement of a 32374 or 18431 gene; 5) an alteration in the level of a messenger RNA transcript of a 32374 or 18431 gene, 6) aberrant modification of a 32374 or 18431 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 32374 or 18431 gene, 8) a non-wild type level of a 32374- or 18431-protein, 9) allelic loss of a 32374 or 18431 gene, and 10) inappropriate post-translational modification of a 32374- or 18431-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 32374- or 18431-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 32374 or 18431 gene under conditions such that hybridization and amplification of the 32374- or 18431-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et al., (1988) *Bio-Technology* 6:1197), or other nucleic acid amplification methods, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

In another embodiment, mutations in a 32374 or 18431 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence

specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 32374 or 18431 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, two-dimensional
5 arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotide probes (Cronin, M.T. et al., (1996) *Human Mutation* 7: 244-255; Kozal, M.J. et al., (1996) *Nature Medicine* 2:753-759). For example, genetic
10 mutations in 32374 or 18431 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. et al., *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations.
15 This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the
20 art can be used to directly sequence the 32374 or 18431 gene and detect mutations by comparing the sequence of the sample 32374 or 18431 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

25 Other methods for detecting mutations in the 32374 or 18431 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al., (1985) *Science* 230:1242; Cotton et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba et al., (1992) *Methods Enzymol.* 217:286-295).

30 In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations

in 32374 or 18431 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al., (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

5 In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 32374 or 18431 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al., (1989) *Proc. Natl. Acad. Sci. USA*: 86:2766, see also Cotton, (1993) *Mutat. Res.* 285:125-144; and Hayashi, (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 32374 or 18431 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al., (1991) *Trends Genet.* 7:5).

15 In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al., (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner, (1987) *Biophys. Chem.* 265:12753).

20 Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki et al., (1986) *Nature* 324:163); Saiki et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:6230).

30 Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides

used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al., (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension
5 (Prossner, (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al., (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany, (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a
10 perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose
15 patients exhibiting symptoms or family history of a disease or illness involving a 32374 or 18431 gene.

Use of 32374 or 18431 Molecules as Surrogate Markers

The 32374 or 18431 molecules of the invention are also useful as markers of
20 disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 32374 or 18431 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the 32374
25 or 18431 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is
30 independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is

difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 32374 or 18431 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a 32374 or 18431 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-32374 or -18431 antibodies may be employed in an immune-based detection system for a 32374 or 18431 protein marker, or 32374- or 18431-specific radiolabeled probes may be used to detect a 32374 or 18431 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of

pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 32374 or 18431 molecules of the invention are also useful as
5 pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By
10 assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 32374 or 18431 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of
15 the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 32374 or 18431 DNA may correlate 32374 or 18431 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

20 Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-32374 or -
18431 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As
25 used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended
30 route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral,

intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a

basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously
5 sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash.

10 Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as
15 magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant,
20 e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic
25 acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention
30 enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release

formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀.

Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such

information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies.

Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al., ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams

per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated.

When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics

(e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 32374 or 18431 expression or activity. Examples of such disorders, e.g., protein kinase-associated or other 32374 or 18431-associated disorders,

include but are not limited to, cellular proliferative and/or differentiative disorders, disorders associated with bone metabolism, immune *e.g.*, inflammatory, disorders, cardiovascular disorders, including endothelial cell disorders, liver disorders, viral diseases, pain or metabolic disorders. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 32374 or 18431 molecules of the present invention or 32374 or 18431 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 32374 or 18431 expression or activity, by administering to the subject a 32374 or 18431 or an agent which modulates 32374 or 18431 expression or at least one 32374 or 18431 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 32374 or 18431 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 32374 or 18431 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Depending on the type of 32374 or 18431 aberrance, for example, a 32374 or 18431, 32374 or 18431 agonist or 32374 or 18431 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some 32374 or 18431 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

As discussed, successful treatment of 32374 or 18431 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 32374 or 18431 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 32374 or 18431 expression is through the use of aptamer molecules specific for 32374 or 18431 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, et al., *Curr. Opin. Chem. Biol.* 1997, 1(1): 5-9; and Patel, D.J., *Curr. Opin. Chem. Biol.* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 32374 or 18431 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of 32374 or 18431 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 32374 or 18431 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 32374 or 18431 through the use of anti-idiotypic antibodies (see, for example, Herlyn, D., *Ann. Med.* 1999;31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A., *Cancer Treat. Res.* 1998;94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 32374 or 18431 protein. Vaccines directed to a disease characterized by 32374 or 18431 expression may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide

sequences encoding single-chain antibodies within the target cell population (see e.g., Marasco et al., (1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or ameliorate 32374 or 18431 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to modulate 32374 or 18431 activity is used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The

subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. et al., (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J., (1994) *Trends in Polymer Science* 2:166-173. Such "imprinted" affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. et al., (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the "free" concentration of compound which modulates the expression or activity of 32374 or 18431 can be readily monitored and used in calculations of IC₅₀.

Such "imprinted" affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC₅₀. A rudimentary example of such a "biosensor" is discussed in Kriz, D. et al., (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 32374 or 18431 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 32374 or 18431 or agent that modulates one or more of the activities of 32374 or 18431 protein activity associated with the cell. An agent that modulates 32374 or 18431 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 32374 or 18431 protein (e.g., a 32374 or 18431 substrate or receptor), a 32374 or 18431 antibody, a 32374 or 18431 agonist or antagonist, a peptidomimetic of a 32374 or 18431 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or 32374 or 18431 activities. Examples of such stimulatory agents include active 32374 or 18431 protein and a nucleic acid molecule encoding 32374 or 18431. In another embodiment, the agent inhibits one or more 32374 or 18431 activities. Examples of such inhibitory agents include antisense 32374 or 18431 nucleic acid molecules, anti-32374 or -18431 antibodies, and 32374 or 18431 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing

the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 32374 or 18431 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) 32374 or 18431 expression or activity. In another embodiment, the method involves administering a 32374 or 18431 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 32374 or 18431 expression or activity.

Stimulation of 32374 or 18431 activity is desirable in situations in which 32374 or 18431 is abnormally downregulated and/or in which increased 32374 or 18431 activity is likely to have a beneficial effect. For example, stimulation of 32374 or 18431 activity is desirable in situations in which a 32374 or 18431 is downregulated and/or in which increased 32374 or 18431 activity is likely to have a beneficial effect. Likewise, inhibition of 32374 or 18431 activity is desirable in situations in which 32374 or 18431 is abnormally upregulated and/or in which decreased 32374 or 18431 activity is likely to have a beneficial effect.

The 32374 or 18431 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, brain disorders, or pain or metabolic disorders as described above, as well as disorders associated with bone metabolism, hematopoietic disorders, liver disorders, viral diseases, heart disorders, blood vessel disorders, and platelet disorders or cardiovascular disorders.

Disorders involving the heart, include but are not limited to, heart failure, including but not limited to, cardiac hypertrophy, left-sided heart failure, and right-sided heart failure; ischemic heart disease, including but not limited to angina pectoris, myocardial infarction, chronic ischemic heart disease, and sudden cardiac death; hypertensive heart disease, including but not limited to, systemic (left-sided) hypertensive heart disease and pulmonary (right-sided) hypertensive heart disease; valvular heart disease, including but not limited to, valvular degeneration caused by calcification, such as calcific aortic stenosis, calcification of a congenitally bicuspid aortic valve, and mitral annular calcification, and myxomatous degeneration of the mitral valve (mitral valve prolapse), rheumatic fever and rheumatic heart disease, infective endocarditis, and noninfected vegetations, such as nonbacterial

thrombotic endocarditis and endocarditis of systemic lupus erythematosus (Libman-Sacks disease), carcinoid heart disease, and complications of artificial valves; myocardial disease, including but not limited to dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and myocarditis; pericardial disease, including but not limited to, pericardial effusion and hemopericardium and pericarditis, including acute pericarditis and healed pericarditis, and rheumatoid heart disease; neoplastic heart disease, including but not limited to, primary cardiac tumors, such as myxoma, lipoma, papillary fibroelastoma, rhabdomyoma, and sarcoma, and cardiac effects of noncardiac neoplasms; congenital heart disease, including but not limited to, left-to-right shunts--late cyanosis, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect, right-to-left shunts--early cyanosis, such as tetralogy of fallot, transposition of great arteries, truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection, obstructive congenital anomalies, such as coarctation of aorta, pulmonary stenosis and atresia, and aortic stenosis and atresia, and disorders involving cardiac transplantation.

Disorders involving blood vessels include, but are not limited to, responses of vascular cell walls to injury, such as endothelial dysfunction and endothelial activation and intimal thickening; vascular diseases including, but not limited to, congenital anomalies, such as arteriovenous fistula, atherosclerosis, and hypertensive vascular disease, such as hypertension; inflammatory disease--the vasculitides, such as giant cell (temporal) arteritis, Takayasu arteritis, polyarteritis nodosa (classic), Kawasaki syndrome (mucocutaneous lymph node syndrome), microscopic polyanglitis (microscopic polyarteritis, hypersensitivity or leukocytoclastic angitis), Wegener granulomatosis, thromboanglitis obliterans (Buerger disease), vasculitis associated with other disorders, and infectious arteritis; Raynaud disease; aneurysms and dissection, such as abdominal aortic aneurysms, syphilitic (luctic) aneurysms, and aortic dissection (dissecting hematoma); disorders of veins and lymphatics, such as varicose veins, thrombophlebitis and phlebothrombosis, obstruction of superior vena cava (superior vena cava syndrome), obstruction of inferior vena cava (inferior vena cava syndrome), and lymphangitis and lymphedema; tumors, including benign tumors and tumor-like conditions, such as hemangioma, lymphangioma, glomus tumor (glomangioma), vascular ectasias, and bacillary angiomatosis, and intermediate-grade (borderline low-grade malignant) tumors, such as Kaposi sarcoma and

hemangioendothelioma, and malignant tumors, such as angiosarcoma and hemangiopericytoma; and pathology of therapeutic interventions in vascular disease, such as balloon angioplasty and related techniques and vascular replacement, such as coronary artery bypass graft surgery.

5 Aberrant expression and/or activity of 32374 or 18431 molecules may mediate disorders associated with bone metabolism. "Bone metabolism" refers to direct or indirect effects in the formation or degeneration of bone structures, e.g., bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and phosphate. This term also includes activities mediated by 32374 or 18431 molecules
10 effects in bone cells, e.g. osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 32374 or 18431 molecules may support different activities of bone resorbing osteoclasts such as the stimulation of differentiation of monocytes and mononuclear phagocytes into osteoclasts. Accordingly, 32374 or 18431 molecules that modulate the production of bone cells can influence bone formation and
15 degeneration, and thus may be used to treat bone disorders. Examples of such disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-convulsant treatment, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary
20 carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk fever.

 Examples of hematopoietic disorders include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis,
25 juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus
30 erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural

hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

Disorders which may be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers. The methods described herein can be used to diagnose or treat hepatocellular necrosis or injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (e.g., bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (e.g., storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (e.g., tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (e.g., Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin, methyl dopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

Additionally, 32374 or 18431 molecules may play an important role in the etiology of certain viral diseases, including but not limited to, Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 32374 or 18431 activity could be used to control

viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, 32374 or 18431 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

5

Pharmacogenomics

The 32374 or 18431 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 32374 or 18431 activity (e.g., 32374 or 18431 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 32374 or 18431 associated disorders (e.g., cellular growth related disorders) associated with aberrant or unwanted 32374 or 18431 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 32374 or 18431 molecule or 32374 or 18431 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 32374 or 18431 molecule or 32374 or 18431 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 and Linder, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high-resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a 32374 or 18431 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 32374 or 18431 molecule or 32374 or 18431 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus

enhance therapeutic or prophylactic efficiency when treating a subject with a 32374 or 18431 molecule or 32374 or 18431 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

5 The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 32374 or 18431 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 32374 or 18431 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells; e.g., cancer cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

15 Monitoring the influence of agents (e.g., drugs) on the expression or activity of a 32374 or 18431 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 32374 or 18431 gene expression, protein levels, or upregulate 32374 or 18431 activity, can be monitored in clinical trials of subjects exhibiting decreased 32374 or 18431 gene expression, protein levels, or downregulated 32374 or 18431 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 32374 or 18431 gene expression, protein levels, or downregulate 32374 or 18431 activity, can be monitored in clinical trials of subjects exhibiting increased 32374 or 18431 gene expression, protein levels, or upregulated 32374 or 18431 activity. In such clinical trials, the expression or activity of a 32374 or 18431 gene, and preferably, other genes that have been implicated in, for example, a 32374- or 18431-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

Other Embodiments

In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with a 32374 or 18431, preferably purified, nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the 32374 or 18431 nucleic acid, polypeptide, or antibody.

The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the 32374 or 18431 nucleic acid, polypeptide, or antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of 32374 or 18431. Such methods can be used to diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder. 32374 or 18431 is associated with protein kinase family members activity, thus it is useful for disorders associated with abnormal lipid metabolism.

The method can be used to detect SNPs, as described above.

In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes:

providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express or mis express 32374 or 18431 or from a cell or subject in which a 32374 or 18431 mediated response has been elicited, e.g., by contact of the cell with 32374 or 18431 nucleic acid or protein, or administration to the cell or subject 32374 or 18431 nucleic acid or protein; contacting the array with one or more inquiry probe, wherein an inquiry probe can be a nucleic acid, polypeptide, or antibody (which is preferably other than 32374 or 18431 nucleic acid, polypeptide, or antibody); providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 32374 or 18431 (or does not express as highly as in the case of the 32374 or 18431 positive plurality of capture probes) or from a cell or subject which in which a 32374 or 18431 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 32374 or 18431 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features, a method of analyzing 32374 or 18431, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 32374 or 18431 nucleic acid or amino acid sequence; comparing the 32374 or 18431 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 32374 or 18431.

Preferred databases include GenBank™. The method can include evaluating the sequence identity between a 32374 or 18431 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for identifying SNP's, or identifying specific alleles of 32374 or 18431. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation

position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with different labels, such that an oligonucleotides which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

Example 1: Identification and Characterization of Human 32374 or 18431 cDNAs

The human 32374 or 18431 sequence (Figure 1A-B; SEQ ID NO:1 or Figure 13A-D; SEQ ID NO:4), which is approximately 2893 or 4136 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1041 or 2682 nucleotides (nucleotides 274-1314 of SEQ ID NO:1; SEQ ID NO:3 or nucleotides 551-3232 of SEQ ID NO:4; SEQ ID NO:6). The coding sequence encodes a 346 or 893 amino acid protein (SEQ ID NO:2 or SEQ ID NO:5).

Example 2: Tissue Distribution of 32374 or 18431 mRNA

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 32374 cDNA (SEQ ID NO:1) or 18431 cDNA (SEQ ID NO:4) can be used. The DNA was radioactively labeled with ³²P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

Example 3: Gene Expression Analysis

Total RNA was prepared from various human tissues by a single step extraction method using RNA STAT-60 according to the manufacturer's instructions (TelTest, Inc). Each RNA preparation was treated with DNase I (Ambion) at 37°C for 1 hour. DNase I treatment was determined to be complete if the sample required at least 38 PCR amplification cycles to reach a threshold level of fluorescence using β -2 microglobulin as an internal amplicon reference. The integrity of the RNA samples following DNase I treatment was confirmed by agarose gel electrophoresis and ethidium bromide staining. After phenol extraction cDNA was prepared from the sample using the SUPERSCRIPT™ Choice System following the manufacturer's instructions (GibcoBRL). A negative control of RNA without reverse transcriptase was mock reverse transcribed for each RNA sample.

Human 32374 or 18431 expression was measured by TaqMan® quantitative PCR (Perkin Elmer Applied Biosystems) in cDNA prepared from a variety of normal and diseased (e.g., cancerous) human tissues or cell lines.

Probes were designed by PrimerExpress software (PE Biosystems) based on the sequence of the human 32374 or 18431 gene. Each human 32374 or 18431 gene probe was labeled using FAM (6-carboxyfluorescein), and the β 2-microglobulin reference probe was labeled with a different fluorescent dye, VIC. The differential labeling of the target gene and internal reference gene thus enabled measurement in same well. Forward and reverse primers and the probes for both β 2-microglobulin and target gene were added to the TaqMan® Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent within a given experiment. A typical experiment contained 200nM of forward and reverse primers plus 100nM probe for β -2 microglobulin and 600 nM forward and reverse primers plus 200 nM probe for the target gene. TaqMan matrix experiments were carried out on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 min at 50°C and 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 sec followed by 60°C for 1 min.

The following method was used to quantitatively calculate human 32374 or 18431 gene expression in the various tissues relative to β -2 microglobulin expression in the same tissue. The threshold cycle (Ct) value is defined as the cycle at which a statistically significant increase in fluorescence is detected. A lower Ct value is indicative of a higher mRNA concentration. The Ct value of the human 32374 or 18431 gene is normalized by subtracting the Ct value of the β -2 microglobulin gene to obtain a Δ Ct value using the following formula: Δ Ct = Ct_{human 59914 and 59921} - Ct _{β -2 microglobulin}. Expression is then calibrated against a cDNA sample showing a comparatively low level of expression of the human 32374 or 18431 gene. The Δ Ct value for the calibrator sample is then subtracted from Δ Ct for each tissue sample according to the following formula: $\Delta\Delta$ Ct = Δ Ct_{sample} - Δ Ct_{calibrator}. Relative expression is then calculated using the arithmetic formula given by $2^{-\Delta\Delta$ Ct}. Expression of the target human 32374 or 18431 gene in each of the tissues tested is then graphically represented as discussed in more detail below.

TaqMan real-time quantitative RT-PCR is used to detect the presence of RNA transcript corresponding to human 32374 relative to a no template control in a panel of human tissues or cells. It is found that the highest expression of 32374 orthologs are expressed in brain tissue as shown in Table 1.

Table 1

Tissue Type	32374	B2.803	Δ Ct	Expression
Adrenal Gland	33.80	19.26	14.54	0.04
Brain	25.67	21.10	4.58	41.96
Heart	38.83	19.16	19.68	0.00
Kidney	39.82	18.97	20.85	0.00
Liver	40.00	19.22	20.79	0.00
Lung	40.00	17.34	22.66	0.00
Mammary Gland	38.74	19.38	19.36	0.00
Pancreas	35.98	22.62	13.36	0.10
Placenta	40.00	20.17	19.83	0.00
Prostate	40.00	19.32	20.68	0.00
Salivary Gland	40.00	20.50	19.51	0.00
Muscle	30.02	22.28	7.74	4.68

	Sm. Intestine	40.00	19.36	20.65	0.00
	Spleen	40.00	17.12	22.88	0.00
	Stomach	40.00	18.80	21.21	0.00
	Teste	32.45	20.42	12.03	0.24
5	Thymus	29.06	18.45	10.61	0.64
	Trachea	40.00	19.45	20.56	0.00
	Uterus	40.00	19.40	20.60	0.00
	Spinal Cord	40.00	19.55	20.45	0.00
	DRG	40.00	19.98	20.03	0.00
10	Skin	40.00	19.17	20.84	0.00

TaqMan real-time quantitative RT-PCR is used to detect the presence of RNA transcript corresponding to 32374 relative to a no template control in a Phase I panel of tissues or cells. It is found that the highest expression of 32374 orthologs are expressed in normal brain cortex tissue as shown in Table 2.

Table 2

	Tissue Type	Mean	β 2 Mean	$\Delta\Delta$ Ct	Expression
	Artery normal	40	23.2	16.8	0
20	Vein normal	40	21.39	18.61	0
	Aortic SMC EARLY	40	22.19	17.81	0
	Coronary SMC	40	23.14	16.86	0
	Static HUVEC	31.97	21.32	10.65	0.6223
	Shear HUVEC	32.46	21.27	11.19	0.4295
25	Heart normal	33.4	19.68	13.72	0.0741
	Heart CHF	29.59	20	9.58	1.3066
	Kidney	33.01	20.96	12.05	0.2358
	Adipose normal	40	21.45	18.55	0
	Pancreas	33.69	22.14	11.55	0.3347
30	primary osteoblasts	40	20.14	19.86	0
	Skin normal	40	22.81	17.19	0
	Spinal cord normal	33.99	21.74	12.26	0.2046

	Brain Cortex normal	26.88	22.3	4.58	41.8102
	Brain Hypothalamus normal	30.16	22.41	7.75	4.6293
	DRG (Dorsal Root Ganglion)	31.9	22.55	9.35	1.5324
	Resting PBMC	29.14	16.94	12.2	0.2133
5	Glioblastoma	30.51	18.86	11.65	0.3101
	Breast normal	34.4	21.3	13.1	0.1139
	Breast tumor	28.25	19.38	8.88	2.1225
	Ovary normal	33.88	21.09	12.8	0.1407
	Ovary Tumor	34.33	21.19	13.14	0.1112
10	Prostate Normal	32.66	20.53	12.13	0.2231
	Prostate Tumor	32.63	19.75	12.88	0.1331
	Colon normal	34.77	19.13	15.64	0.0196
	Colon Tumor	32	19.76	12.24	0.2067
	Lung normal	36.75	19.06	17.69	0
15	Lung tumor	27.7	19.51	8.19	3.4361
	Lung COPD	33.56	19.18	14.38	0.0469
	Colon IBD	34.81	18.47	16.34	0.012
	Liver normal	37.17	20.94	16.23	0
	Liver fibrosis	37.07	22.65	14.42	0
20	Dermal Cells- fibroblasts	40	20.04	19.97	0
	Spleen normal	34.63	20.96	13.66	0.077
	Tonsil normal	31	18.02	12.98	0.1233
	Lymph node	30.9	19.35	11.55	0.3347
	small Intestine	36.72	20.86	15.86	0
25	Skin-Decubitus	36.12	21.31	14.82	0
	Synovium	38.9	20.3	18.59	0
	BM-MNC (Bone marrow mononuclear cells)	28.93	17.2	11.73	0.2954
	Activated PBMC	31.73	18.6	13.13	0.1116
30	Skeletal Muscle	29.23	22.68	6.55	10.6722
	Osteoclasts (diff)	38.66	18.39	20.27	0.0008
	Nerve	38.6	22.86	15.74	0.0182

Epithelial Cells (Prostate) 38.72 25.25 13.47 0.0881

TaqMan real-time quantitative RT-PCR is used to detect the presence of RNA transcript corresponding to rat 32374 relative to a no template control in a Phase I panel of tissues or cells. It is found that the highest expression of 32374 orthologs are expressed in rat adrenal gland, as shown in Table 3.

Table 3

	Tissue	r32374	18S	ΔCt	Expression
10	Brain	24.32	12.46	11.86	0.27
	Spinal Cord	27.01	12.84	14.17	0.05
	DRG	25.85	13.29	12.56	0.17
	SCG	29.57	13.25	16.32	0.01
	Hairy Skin	29.39	13.67	15.72	0.02
15	Gastro Muscle	28.88	14.00	14.88	0.03
	Heart	27.14	12.90	14.24	0.05
	Kidney	29.06	13.00	16.07	0.01
	Liver	27.72	12.59	15.13	0.03
	Lung	30.27	12.45	17.82	0.00
20	Spleen	27.90	13.59	14.31	0.05
	Aorta	28.26	13.62	14.64	0.04
	Adrenal Gland	24.22	13.17	11.05	0.47
	Salivary Gland	29.10	13.00	16.10	0.01
	Thyroid	30.28	13.93	16.35	0.01
25	Prostate	31.23	13.23	18.00	0.00
	Thymus	25.87	13.28	12.60	0.16
	Trachea	29.92	13.85	16.07	0.01
	Esophagus	27.32	13.87	13.45	0.09
	Duodenum	32.78	14.31	18.47	0.00
30	Diaphragm	25.70	13.42	12.28	0.20
	Colon	29.27	14.99	14.28	0.05

Expression of 32374 was also detected in a phase II panel of DRG (dorsal root ganglion) tissues and cell lines as shown in Table 4.

Table 4

	Tissue	r32374	18S	δCt	Expression
5	Naïve DRG	24.91	11.41	13.50	0.09
	I DRG CCI 3	24.96	11.56	13.41	0.09
	I DRG CCI 7	25.16	11.58	13.58	0.08
	I DRG CCI 10	25.03	11.35	13.68	0.08
10	I DRG CCI 28	24.38	11.49	12.89	0.13
	Naïve DRG	24.72	11.45	13.27	0.10
	I DRG CFA 1	25.22	11.55	13.67	0.08
	I DRG CFA 3	25.41	11.45	13.96	0.06
	I DRG CFA 7	25.34	11.59	13.75	0.07
15	I DRG CFA 10	25.20	11.60	13.60	0.08
	I DRG CFA 14	24.70	11.37	13.33	0.10
	I DRG CFA 28	24.79	11.37	13.43	0.09
	Naïve DRG	24.86	11.30	13.56	0.08
	I DRG AXT 1	24.82	11.35	13.47	0.09
20	I DRG AXT 3	25.20	11.53	13.67	0.08
	I DRG AXT 7	25.09	11.39	13.70	0.08
	I DRG AXT 14	25.00	11.47	13.54	0.08

Expression of 32374 was also detected in a phase III panel of SC tissues and cell lines as shown in Table 4. The level of expression in all tissue and cell line samples was higher than in naïve tissue and cell line samples.

Table 5

	Tissue	r32374	18S	δCt	Expression
30	Naïve SC	26.74	11.44	15.30	0.03
	I SC CCI 3	25.86	11.62	14.24	0.05
	I SC CCI 7	26.01	12.05	13.96	0.06

	I SC CCI 10	25.66	12.26	13.40	0.09
	I SC CCI 14	25.52	11.64	13.88	0.07
	I SC CCI 28	25.74	12.19	13.55	0.08
	Naïve SC	26.92	11.53	15.39	0.02
5	I SC CFA 1	26.31	11.89	14.41	0.05
	I SC CFA 3	26.03	11.52	14.52	0.05
	I SC CFA 7	26.34	11.52	14.82	0.04
	I SC CFA 10	25.89	11.54	14.35	0.05
	I SC CFA 14	26.45	12.21	14.24	0.05
10	I SC CFA 28	26.48	12.26	14.23	0.05
	Naïve SC	27.18	11.45	15.73	0.02
	I SC AXT 1	26.50	11.77	14.73	0.04
	I SC AXT 3	26.45	11.69	14.76	0.04
	I SC AXT 7	26.42	11.81	14.61	0.04
15	I SC AXT 14	25.94	12.61	13.32	0.10

Expression of 18431 was detected in an oncology phase panel as shown in Table 6 and shows highest relative expression in a breast tumor sample, higher expression in normal ovary compared to ovary tumor tissue or cell samples, and an upregulation in lung tumor compared to normal lung tissue or cell samples.

Table 6

		Average	Average	Relative
	Breast N	30.5	22.8	19.3
25	Breast N	31.5	21.4	3.7
	Breast N	28.1	17.5	2.7
	Breast N	27.7	19.7	16.9
	Breast T	26.5	17.7	8.9
	Breast T	26.2	18.1	15.1
30	Breast T	24.9	17.1	17.7
	Breast T	26.1	17.2	8.3
	Breast T	27.8	18.9	8.5

	Breast T	25.1	20.2	137.3
	Ovary N	24.1	18.3	73.0
	Ovary N	24.9	19.4	88.1
	Ovary N	26.4	19.7	39.1
5	Ovary N	28.3	22.8	87.8
	Ovary T	26.9	18.9	15.6
	Ovary T	25.6	18.2	25.3
	Ovary T	25.0	17.3	19.2
	Ovary T	26.1	18.3	18.5
10	Ovary T	25.9	17.7	14.0
	Ovary T	28.3	19.6	10.5
	Ovary T	27.5	20.7	38.6
	Ovary T	27.2	17.1	3.9
	Lung N	28.0	17.2	2.3
15	Lung N	31.8	19.2	0.6
	Lung N	26.1	16.6	5.5
	Lung N	28.3	16.3	1.0
	Lung T	23.5	16.5	31.1
	Lung T	23.8	17.3	46.7
20	Lung T	25.7	18.2	21.9
	Lung T	25.0	17.0	16.1
	Lung T	25.6	19.2	46.6
	Lung T	25.6	19.3	52.6
	Lung T	24.5	17.9	42.7
25	H460-p16 24	26.3	16.3	4.3
	H460-p16 48	26.1	17.0	7.5
	H460-p16 72	25.9	16.5	5.9
	H460-p16 96	26.1	17.3	8.9
	H460+p16 24	25.8	17.3	11.5
30	H460+p16 48	26.2	16.8	5.8
	H460+p16 72	25.8	16.8	8.5
	H460+p16 96	26.6	16.7	4.2

Expression of 18431 was also detected in a second oncology phase panel as shown in Table 7 and shows highest relative expression in a normal brain sample, and higher expression in normal brain compared to brain tumor tissue or cell samples.

5

Table 7

		Average	Average	Relative
	Colon N	28.8	21.2	5.3
	Colon N	26.3	20.8	22.4
10	Colon N	27.2	18.6	2.6
	Colon T	24.3	17.5	8.4
	Colon T	28.1	19.4	2.4
	Colon T	24.1	17.0	7.2
	Colon T	25.5	19.1	11.9
15	Colon T	26.7	17.5	1.8
	Colon T	24.6	18.0	10.9
	Liver Met	25.7	18.6	7.5
	Liver Met	26.1	21.2	33.6
	Liver Met	24.7	19.9	35.5
20	Liver Met	25.1	19.0	14.7
	Liver Nor	25.0	17.7	6.6
	Liver Nor	29.2	25.0	55.6
	Brain N	25.0	21.4	83.6
	Brain N	25.2	22.4	145.6
25	Astrocyt	26.9	23.1	72.3
	Brain T	24.7	17.7	7.8
	Brain T	23.5	17.3	13.7
	Brain T	26.2	18.5	4.8
	Brain T	24.4	18.7	18.5
30	Brain T	28.1	19.6	2.7
	HMVEC-Arr	23.9	17.4	11.7
	HMVEC-Prol	23.8	18.5	25.5

	Placenta	28.1	23.6	42.1
	Fetal Adrenal	29.9	24.4	23.0
	Fetal Adrenal	30.3	26.7	80.5
	Fetal Liver	25.3	21.2	60.2
5	Fetal Liver	25.6	20.0	21.2

Example 4: Recombinant Expression of 32374 or 18431 in Bacterial Cells

In this example, 32374 or 18431 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 32374 or 18431 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-32374 or -18431 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 5: Expression of Recombinant 32374 or 18431 Protein in COS Cells

To express the 32374 or 18431 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 32374 or 18431 protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 32374 or 18431 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 32374 or 18431 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 32374 or 18431 coding sequence. The PCR amplified fragment and the

pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA).

Preferably the two restriction sites chosen are different so that the 32374 or 18431 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 α , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 32374- or 18431-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 32374 or 18431 polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 32374 or 18431 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 32374 or 18431 polypeptide is detected by radiolabelling and immunoprecipitation using a 32374 or 18431 specific monoclonal antibody.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated 32374 or 18431 nucleic acid molecule selected from the group consisting of:

- 5 a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
- 10 b) a nucleic acid molecule comprising a fragment of at least 15 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
- 15 c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
- 20 d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
- 25 e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or a complement thereof, under stringent conditions;
- 30 f) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____; and

g) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____.

2. The isolated nucleic acid molecule of claim 1, which is the nucleotide sequence SEQ ID NO:1 or SEQ ID NO:4.

3. A host cell which contains the nucleic acid molecule of claim 1.

4. An isolated 32374 or 18431 polypeptide selected from the group consisting of:

a) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or a complement thereof under stringent conditions;

c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:5; and

d) the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5.

5. An antibody which selectively binds to a polypeptide of claim 4.

6. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;

5 b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;

10 c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID
15 NO:6; and

d) the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5;
comprising culturing the host cell of claim 3 under conditions in which the nucleic acid molecule is expressed.

7. A method for detecting the presence of a nucleic acid molecule of claim 1 or
20 a polypeptide encoded by the nucleic acid molecule in a sample, comprising:

a) contacting the sample with a compound which selectively hybridizes to the nucleic acid molecule of claim 1 or binds to the polypeptide encoded by the nucleic acid molecule; and

b) determining whether the compound hybridizes to the nucleic acid or binds to
25 the polypeptide in the sample.

8. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 or binds to a polypeptide encoded by the nucleic acid molecule and instructions for use.

9. A method for identifying a compound which binds to a polypeptide or modulates the activity of the polypeptide of claim 4 comprising the steps of:

a) contacting a polypeptide, or a cell expressing a polypeptide of claim 4 with a test compound; and

5 b) determining whether the polypeptide binds to the test compound or determining the effect of the test compound on the activity of the polypeptide.

10. A method for modulating the activity of a polypeptide of claim 4 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the
10 polypeptide.

11. A method of identifying a nucleic acid molecule associated with a disorder comprising:

a) contacting a sample from a subject with or at risk of developing a disorder comprising nucleic acid molecules with a hybridization probe comprising at least 25
15 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4 defined in claim 2; and

b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a nucleic acid molecule associated with a disorder.

12. A method of identifying a nucleic acid associated with a disorder
20 comprising:

a) contacting a sample from a subject having a disorder or at risk of developing a disorder comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4 defined in claim 2 and the second primer comprising at least 25 contiguous
25 nucleotides from the complement of SEQ ID NO:1 or SEQ ID NO:4;

b) incubating the sample under conditions that allow nucleic acid amplification; and

c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying the nucleic acid molecule associated with a disorder.

13. A method of identifying a polypeptide associated with a disorder comprising:

a) contacting a sample comprising polypeptides with a 32374 or 18431 binding partner of the 32374 or 18431 polypeptide defined in claim 4; and

5 b) detecting the presence of a polypeptide in the sample that binds to the 32374 or 18431 binding partner, thereby identifying the polypeptide associated with a disorder.

14. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

10 a) contacting a sample obtained from the subject comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4 defined in claim 2; and

b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a subject having a disorder or at risk for developing a disorder.

15 15. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

a) contacting a sample obtained from the subject comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:4 defined in claim 2 and
20 the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1 or SEQ ID NO:4;

b) incubating the sample under conditions that allow nucleic acid amplification; and

25 c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying a subject having a disorder or at risk for developing a disorder.

16. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

a) contacting a sample obtained from the subject comprising polypeptides with a 32374 or 18431 binding partner of the 32374 or 18431 polypeptide defined in claim 4;
and

b) detecting the presence of a polypeptide in the sample that binds to the 32374 or 18431 binding partner, thereby identifying a subject having a disorder or at risk for developing a disorder.

17. A method for identifying a compound capable of treating a disorder characterized by aberrant 32374 or 18431 nucleic acid expression or 32374 or 18431 polypeptide activity comprising assaying the ability of the compound to modulate 32374 or 18431 nucleic acid expression or 32374 or 18431 polypeptide activity, thereby identifying a compound capable of treating a disorder characterized by aberrant 32374 or 18431 nucleic acid expression or 32374 or 18431 polypeptide activity.

18. A method for treating a subject having a disorder or at risk of developing a disorder comprising administering to the subject a 32374 or 18431 modulator of the nucleic acid molecule defined in claim 1 or the polypeptide encoded by the nucleic acid molecule or contacting a cell with a 32374 or 18431 modulator.

19. The method of claim 18, wherein the 32374 or 18431 modulator is

- a) a small molecule;
- b) peptide;
- c) phosphopeptide;
- d) anti-32374 or 18431 antibody;
- e) a 32374 or 18431 polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or a fragment thereof;

- f) a 32374 or 18431 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4; or

g) an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or SEQ ID NO:4 at 6X SSC at 45°C, followed by one
5 or more washes in 0.2X SSC, 0.1% SDS at 65°C.

20. The method of claim 18, wherein the 32374 or 18431 modulator is

- a) an antisense 32374 or 18431 nucleic acid molecule;
- b) is a ribozyme;
- c) the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:4, or a fragment

10 thereof;

d) a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of
15 12, and a gap penalty of 4;

e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or SEQ ID NO:4 at 6X SSC at 45°C, followed by one
20 or more washes in 0.2X SSC, 0.1% SDS at 65°C; or

f) a gene therapy vector.

21. A method for evaluating the efficacy of a treatment of a disorder, in a subject, comprising:

treating a subject with a protocol under evaluation;

25 assessing the expression level of a 32374 or 18431 nucleic acid molecule defined in claim 1 or 32374 or 18431 polypeptide encoded by the 32374 or 18431 nucleic acid molecule,

wherein a change in the expression level of 32374 or 18431 nucleic acid or 32374 or 18431 polypeptide after the treatment, relative to the level before the treatment, is
30 indicative of the efficacy of the treatment of a disorder.

22. A method of diagnosing a disorder in a subject, comprising:
evaluating the expression or activity of a 32374 or 18431 nucleic acid molecule defined in
claim 1 or a 32374 or 18431 polypeptide encoded by the 32374 or 18431 nucleic acid
molecule, such that a difference in the level of 32374 or 18431 nucleic acid or 32374 or
5 18431 polypeptide relative to a normal subject or a cohort of normal subjects is indicative
of a disorder.

23. The method defined in claim 18, wherein the disorder is cancer or aberrant
cellular proliferation and/or differentiation, a pain or metabolic disorder, or a brain
10 disorder.

24. The method defined in claim 23, wherein the cancer or aberrant cellular
proliferation and/or differentiation is lung, ovarian, or brain cancer.

ACGCAACTTCGGGCCAGGGGTCCGCCCCAAGGGGTCCGAAGAGCCTTGAACCGGCCCGGCTCCTTGACCTTGCTTGT SEQ ID NO:1

GGGCCGAAGGACTGCCCCCTGGGCCTGGGTCCGGTGTGCCCCCTTCTCACTTGAAGACA..CAGGCCCTGACTCTCCG

CACACTTGGCCCGCCAGCGACGTCAACCAAGCACCTACGAAACTAGTCCCGGGAGCTGGGCAAAGGCACCTACGGGAAG

GTGATCTGGTGGTCTACAAGGGCACAGGCACAAA	M A L K F V N K S K	10
ATG GCA CTG AAG TTT GTG AAC AAG AGC AAA		SEQ ID NO:2
↑SEQ ID NO:3		30
T K L K N F L R E V S I T N S L S S S P		30
ACC AAG CTG AAG AAC TTC CTA CGG GAG GTG AGC ATC ACC AAC AGC CTC TCC TCC AGC CCC		90
F I I K V F D V V F E T E D C Y V F A Q		50
TTC ATC ATC AAG GTC TTT GAC GTG GTC TTT GAG ACA GAG GAC TGC TAC GTC TTT GCC CAG		150
E Y A P A G D L F D I I P P Q V G L P E		70
GAG TAC GCA CCT GCT GGG GAC CTG TTT GAC ATC ATC CCT CCC CAG GTG GGG CTC CCT GAG		210
D T V K R C V Q Q L G L A L D F M H G R		90
GAC ACC GTG AAG CGC TGT GTG CAG CAG CTG GGC CTG GCG CTG GAC TTC ATG CAC GGG CGG		270
Q L V H R D I K P E N V L L F D R E C R		110
CAG CTG CTG CAC CGC GAC ATC AAG CCC GAG AAC GTG CTG CTG TTC GAC CGC GAG TGC CGC		330
R V K L A D F G M T R R V G C R V K R V		130
CGC GTA AAG CTG GCC GAC TTC GGC ATG ACG CGC CGC GTG GGC TGC CGC GTC AAG CGC GTG		390
S G T I P Y T A P E V C Q A G R A D G L		150
AGC CGC ACC ATC CCT TAC ACG GCG CCT GAG GTG TGC CAG GCG GGC CGC GCC GAC GGG CTG		450
A V D T G V D V W A F G V L I F C V L T		170
GCG GTG GAC ACG GGC GTG GAC GTG TGG GCC TTC GGC GTG CTC ATC TTC TGC GTG CTC ACC		510
G N F P W E A A S G A D A F F E E F V R		190
GGC AAC TTC CCG TGG GAG GCG GCG TCG GGC GCC GAC GCC TTC TTC GAG GAG TTC GTG CGC		570
W Q R G R L P G L P S Q W R R F T E P A		210
TGG CAG CGG GGC CGC CTG CCG GGG CTG CCT TCG CAG TGG CGC CGC TTC ACC GAG CCC GCG		630
L R M F Q R L L A L E P E R R G P A K E		230
CTG CGC ATG TTC CAG CGC TTA CTG GCC CTG GAG CCC GAG CGC CGC GGC CCA GCC AAG GAG		690
V F R F L K H E L T S E L R R R P S H R		250
GTG TTC CGC TTC CTC AAG CAC GAG CTC ACG TCC GAG CTG CGC CGC CGG CCC TCG CAC CGC		750
A R K P P G D R P P A A G P L R L E A P		270
GCG CGC AAG CCC CCC GGG GAC CGC CCG CCC GCC GCC GGG CCA CTG CGC CTC GAG GCG CCT		810
G P L K R T V L T E S G S G S R P A P P		290
GGG CCG CTC AAG CGG ACG GTG CTG ACC GAG AGC GGC AGC GGC TCC CGG CCC GCG CCC CCC		870
A V G S V P L P V P V P V P V P V P		310
GCC GTC GGG TCG GTG CCC TTG CCC GTG CCG GTG CCG GTG CCA GTG CCC GTG CCG GTG CCT		930

FIG 1A

V	P	E	P	G	L	A	P	Q	G	P	P	G	R	T	D	G	R	A	D	330
GTG	CCC	GAG	CCC	GGC	CTA	GCT	CCC	CAG	GGG	CCC	CCC	GGC	CGG	ACC	GAC	GGC	CGC	GCG	GAC	990
K	S	K	G	Q	V	V	L	A	T	A	I	E	I	C	V	*				347
AAG	AGC	AAA	GGG	CAG	GTG	GTG	CTG	GCC	ACG	GCC	ATC	GAG	ATC	TGC	GTC	TGA				1041

←SEQ ID NO:3↑

GTCGCCTCCGCCGCCCTCGGACCCGGGAGCAGCCCGGGCCCCGCCCGAGCCGGTGCCCGGTGCGGCGGTAGGGAATGGA
 GCCACCTCGCCGCGGGCAGGGGGCGCAGCGGTAGACTAGGCAGGACGCGGCCCGGCACCTGGTCCGTCCCCGGCGGGC
 TGGTGAGGGGGCCACCAAAGACCCCTA3CGCGGCCTGGTGAGCGGGGGCTTGGCCCAGAGGAGCCAAGCCGCACAGACC
 CGAGAATTTCGGAGGCGACCACACAACACACACACACACATACACACACACACACACACAGCCAGGAGCAAGGGAGC
 TTTCGGGCCACACTCCAGACGCCTCCCTGAGCCCTGGAACCCGGACTCGTTGCTCCTGGCCTTCCATACCCCTGGCA
 GATCATCCTGCGGTCCACCCCAGATCCCTCCTCCTCGCCATCCCATCTGCCCCCTCCCCACCCTGGGTACAGAAAG
 GGACTGAAGTGTTGGGCAGAGAGGGGGCTTAAGGCCCTTGGGCACAGGCTGGGATCAGGGCAGTGAGCGAAGGGCAGCT
 GTGTCTGCCCTTCCTTCTGGAGGCTGGAGGGGAGAGGCCAAGCCCTTGGAAAATGTAGCAAATGTCTGGRWKGTGCGA
 TAAGTGCGTGATGTGCGGGACAGGCCCCGAGAAGCTAGTGACTCCTGCACACCCCCATTGCACAAATGAAATCACAGC
 CCAGGAGGGAGGGTAGCTTGGCACTGGCTGAGAAATAGAGCTCTCTCCCCGCCCTCCCCCTAACCACAAGGGATTGTCT
 CTGACAACTTGTGGGGATAGAAGGGCTCACAGGGCAGGGGTCTCAGCTGCCCCATCCTTAGGGCAGGGGAGTTAGTGT
 GGAGCCGAGAGCAGGTCCCAGCTCCCCCTGCCAGCCGCACTGTCCAGGGCCAGGGACCTCTGCCGGGTCTCCAGCC
 CTTGCCACACAGCCTAGACGTAGTAGCCTGGGCTTCAGCAGGTGGCGAGCTGGTTCTGTCTGGAAATTTCTCCTGGGT
 TTCTTGGGGTCAAACATGCCAACCTCCAAGACCCCATCTCACGTCTCCCACTTTTCTGGCGCTGGAGTGTGCAGGGCG
 TAGGACCTGCATGTGTGGGTGTGAGAATGGGGCCGGTGGACACCAGGGGGCAGTGTGTGACTAGGTGTGTGTGCACA
 TGTGTAGGGTGCAGACGCATGGGTGCCATCCTTTGCNTTCAATGACTGTGCGTCCAGACCCCAAAAAGCGCCCCCCC
 ACCACACCCTGNTCCTCCCAGGCAGCTGTCCCAGGGCGCCAGGCCTGCCTTGACCCACAGCCCTCAGGAAATCCGGCA
 AGGAGGCCCCCTGCAGGTGGTTTCANGCCAGGTAGCAAAACAGAGACAACAGCAGCCCCGCCTGACCCCTGCCCTNT
 CTGTGGAGGCCCCGGACCCCGCAATAAGCACCATGGGTGAGGCTGTCCCTGTGAGGNNCCCTGCCAGGTCCCTC
 CTGGGGTTCTGGGCCATTTGAGGGGCTCTTTGATGGGCCAGGCCNGCCAGAGTGAACCTCCGAGCACTTTCTGGCTGGT

FIG 1B

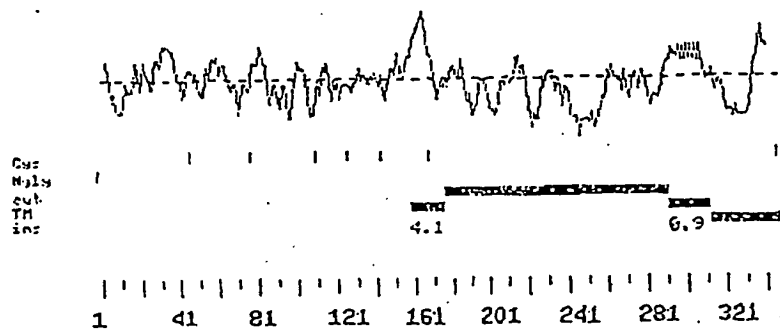


Fig. 2


```

*->vAvKilkkesls....lrEiqilkrIs.HpNIvrlIgvfedtdhly  SEQ ID NO:7
  +A+K ++k++++ ++ lrE++i ++ls+ p+I++++v+ +t+d +
32374  1  MALKFVNKSKTKlknfLREVSITNSLSsSPFIiKVFDVVFETEDCYV 47

  lvmEymegGdLfdylrrngplsekeakkialQilrGleYlHsngivHRDL
  + +Ey++ GdLfd++ + l+e+ +k+++ Q+ +l+++H++ VHRD+
32374  48  FAQEYAPAGDLFDIIPPQVGLPEDTVKRCVQQLGLALDFMHGRQLVHRDI 97

  KpeNILlden..gtvKiaDFGLArll.eklTtfvGTpwYmmAPEvileg.
  KpeN+Ll +++ vK+aDFG+ r ++ + + + GT++Y APEv + +
32374  98  KPENVLLFDRcRRVKLADFGMTRRVgCRVKRVSGTIPYT-APEV-CQAg 145

  ....rgysskvDvWSlGviLyElltggplfpgadlpaftggdevdqliif
  + ++ ++ vDvW++Gv+++ +ltg
32374  146  radgLAVDGTGVDVWAFGLIFCVLTG----- 171

  vlklPfsdelpktridpleelfrikkr.....rlplpsncSeelkdL
  P++ + ++ ++ f+ r ++++ ++++ ++++ e+++
32374  172  --NFPWEAA-----SGADAFFEEFVRwqgrlpgLPSQWRRFTEPALRM 213

  lkkcLnkDPskRpGsatakei<-*
  ++L++ P++R ake+
32374  214  FQRLLALEPERRG---PAKEV 231

```

Fig. 3

```

Query:  226  GPAKEVFRFLKHELTSELRRRPSHRARKPPGDRPPAAGPLRLEAPGPLKRTVLTESGSGS 285
        GPAKEVFRFLKHELTSELRRRPSHRARKPPGDR P  GPLRLEAPGPLKRTVLTESGSGS
Sbjct:  1  GPAKEVFRFLKHELTSELRRRPSHRARKPPGDRLP--GPLRLEAPGPLKRTVLTESGSGS 58  SEQ ID NO:8

Query:  286  R 286
        R
Sbjct:  59  R 59

```

Fig. 4

```

Query:  321  PPGRTDGRADKSKGQVVLATAIEICV 346
        PPGRTDGRADKSKGQVVLATAIEICV
Sbjct:  89  PPGRTDGRADKSKGQVVLATAIEICV 114  SEQ ID NO:9

```

Fig 5

```

Query:  166  FCVLtGNFPWEAASGADAFFEEFVRWQRGLPGLPSQWRRFTEPALRMFQRLLALEPERR 225
        +C + G FPW+ AS + E+ +W + + P LP ++ F+E AL++F++ L + R
Sbjct:  3  YC-MKGKFPWQKASIMCKPYWEWEQWLKRKNPALPKKFNPFSEKALKLFKKSltPRFKDR 61  SEQ ID NO:10

Query:  226  GPAKEVFRFL-KHELTSELRR 245
        AK++ + L K +L ++R
Sbjct:  62  WTAKDMRKCLAKEKLLKSVKR 82

```

Fig. 6

Query: 4 K F V N K S K T K L K N F L R E V S I T N S L S S S P F I I K V F D V V F E T E D C Y V F A Q E Y A P A G D L F D I I P 63
 K V S K + + L E + + L + + F + I + + + Y + + Y + + + I
 Sbjct: 16 K M V A F S K R E E E R I L L E I D L Y K K L E N N E F V I D L M A H I V D D I T H Y L L F D K Y S Q -- N F L E Y I E 73 SEQ ID NO:11

Query: 64 P - Q V G L P E D T V K R C V Q Q L G L -- A L D F M H G R Q L V H R D I K P E N V L L F D R E C R R V K L A D F G 118
 + + G D + K G + A + + + H G + H D I K P N + L + + K + D F G
 Sbjct: 74 E L K I G G E V D E L K H L K Y F S G I V S A I E Q L H G F E F A H L D I K P A N I L --- K S G D T I K M I D F G 128

Fig. 7

Query: 47 V F A Q E Y A P A G D L F D I I P P Q V G L P E D T V K R C V Q ----- Q L G L A L D F M H ----- G R Q 91
 + A E + P G L D + V + D + + + Q Q + A L + + H G + +
 Sbjct: 175 M I A L E W L P G G T L A D Y F Q F K V R E K D S E R S P I Q L K D M L S I L Y Q V S Q A L K Y I H S Q L D E F G Q E 234 SEQ ID NO:12

Query: 92 L V H R D I K P E N V L L F D R E C R R -- V K L A D F G M T R R V G C R V K R V S G T I P Y T A P E V - C Q A G R A D 148
 L H I N V L + + + R + V K L D F G + G + I Y P E + C A R
 Sbjct: 235 L T H G R I F T R N V L V T E P D L R K C E V K L G D F G - D A P M G L E Y S -- T P I I A Y M P P E I L C C A E R I P 291

Query: 149 G L A V D T G V D V W A F G V L I F - C V L T G N F P 174
 + D V W F G V I + C + G P
 Sbjct: 292 P H R P E N -- D V W M F G V F I W E C L T L G A Q P 316

Fig. 8

Query: 77 V Q Q L G L A L D F M H G R Q L V H R D I K P E N V L L --- F D R E C R R V K L A D F G M T R --- R V G C R V K 128
 + Q L A + + H + + R D + K + N + L L F D E + + + A D F G + V
 Sbjct: 319 I A Q L E A C T Y L H K H K V A Q R D M K S D N I L L E Y D F D E I P Q L V V A D F G C A L A C D N W Q V D Y E S D 378 SEQ ID NO:13

Query: 129 R V S -- G T I P Y T A P E V C Q A G R A D G L A V D T G V - D V W A F G V L I F C V L T G N F P W E A A S G A D A F F 185
 V S G A P E + A + V + + D W A G L + V L T + P +
 Sbjct: 379 E V S L G G N A K T K A P E I A T A V P G K N V K V N F E M A D T W A A G G L S Y E V L T R S N P F Y K L L ----- 432

Query: 186 E E F V R W Q R G R L P G L P S Q 202
 + + Q L P L P S +
 Sbjct: 433 - D T A T Y Q E S E L P A L P S R 448

Fig. 9

Query: 68 L P E D T V K R C V Q Q L G L A L D F M H G R Q L V H R D I K P E N V L L F D R -- E C R R V K L A D F G M T R R V G C 125
 L P D + + L A + D F + G + H R D I K P + N + + R R + L D F + G
 Sbjct: 647 L P V D Q L E A Y G D Y L F G A V D F L E G E G I W H R D I K P D N I A V R I R P N R T R E L V L I D F S L A --- G Y 703 SEQ ID NO:14

Query: 126 R V K R V - S G T I P Y T A P E V C Q A G R A D G L A V D T G V D V W A F G V L I F C V L T G N F P - W E A A S G A D A 183
 K + G T Y P V R + D + + A V + + G P W S
 Sbjct: 704 P A K N T D A G T D G Y L D P F V D V I T R G --- S Y D S H A E R Y A V A V T L H Q M A S G E L P K W G D G S V L P R 760

Query: 184 F F E E F V R W Q R G R L P G L P S Q W R R F T E P A L R ----- M F Q R L L A L E P E R R G P A K E V F R -- F L K 236
 + W P + + + F + P A + R F Q + L + + R P + R + K
 Sbjct: 761 M T D P - K E W P --- Y P T I A A E -- A F - D P A V R D G L V A F F Q K A L H R D A G K R F E L K P M R D A W R K 813

Query: 237 H E L T S E L R R R P S H R A R K P - P G D - R P P A A G 263
 L + S H R R P D P A G
 Sbjct: 814 V F L D A S Q T V P S S H R T R P A A P A D G A A P A E G 842

Fig. 10

Query: 30 PFIIKVFDDVVFETEDCYVFAQEYAPAGDLFDIIPPQVGLP-----EDTVKRCVQQLGLALD 85
 P I + + +V E + C++ QE G + + G+P E+ + +QQL LD
 Sbjet: 71 PGILAIENVSEEDRCFLVTQEND--GPILSLTQYLKGIPRKLTEEEIVDIIQQLCSLLD 128 SEQ ID NO:15

Query: 86 FMHGRQLVHRDIKPENVLL-FDRECRVKLADFGMTRRVGCR-----VKKRV 130
 ++H L H +V + F + L D G + R +++++
 Sbjet: 129 YVHSEGLAHGQWNLSVHIHFLNGVPNILPDLGFASLIRERMFDFGMQDEENRESIEKI 188

Query: 131 SGTIPYTAPEVCQAGRADGLAVDTGVDVWAFGVLIFCVLTGNFPWEAASGADAFEEFV 189
 + + PE Q +G DT +AFG + + +L G FPW F +F+
 Sbjet: 189 RDRLLFHTPEGKQT---NGRETD---YAFGAITYYLLFGFFPWGIFPKPSKCFPDFI 240

Fig. 11

Query: 29 SPFIKVFDDVVFETEDC-YVFAQEYAPAGDLFDIIPPQVGLPEDTVKRCVQQLGLALDFM 87
 SP ++ V D++ E E VF E L +++ PE ++ L L
 Sbjet: 82 SPHVLPVRDLIDEGEWSLVF--EPRRTITLRELLSAGPVSPE-LLQPLTTALFEGLSAA 138 SEQ ID NO:16

Query: 88 HGRQLVHRDIKPENVLLFDRECRVKLADFGMTRRVGCRVKKVSGTIP-YTAPEVCQAGR 146
 H L+H I PE V FD + +R LA+FG+ RR ++ P Y APE+ G
 Sbjet: 139 HQGALLHTQISPEAVW-FDTQ-KRPLLAEFGLARRTAQELRDHWPDPYAAPELLSGG- 195

Query: 147 ADGLAVDTGVDVWAFGVLIFCVLTGNFPWEAASGADAFEEFVRWQGRLP-----GLPSQ 202
 D++A + EAA+G A R Q RLP G+P Q
 Sbjet: 196 ----PYTPQTDLYALAATLL-----EAATGT-ALSPVSARQQGVRLPSWPAGIPPQ 241

Query: 203 WRRFTEPALRMFQRLLEPERRGPAKEVFRFLKH-ELTSELRRRPSHRARKPPGDRPPA 261
 E L++ + A+ A EV L+ + T + + A P PPA
 Sbjet: 242 VAHALESCLQLDPAVRAVS-----AAEVLEELRRAOPTQAILSQQEPPAPPPSVPSPPA 295

Query: 262 A 262
 A
 Sbjet: 296 A 296

Fig. 12

CTTCCTCTTCTGTGCTCAGTCCCATTAACTGCCATACCACGGCTCCTCGTCTTCCCAATTCCCTCAGTATTTT SEQ ID NO:4

CAATCGACCCCCCGTCCCCCGCACCTCTTCTCTCTCGCTATATGTCTTTCGTGGCCAGTTTGGGCAAGGGGAA

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AGGGTAGAGGGTGAAAAGCTCGGATCTGTGTTTGGGGAAGGCCAGGCTTGCGCTCCTCGCCGGGTTCCGCGAAGGTTAA

CCTTGGCTGACTTGGCTCGCGAGCAAAGGGCAGCGTCTGAGCTCCCGCGGTTCCAGGAGTGGCCTCTTTGTAGGAGCA

CCTGAAATGCAGCGTCTGGTGACTAAGCCGTAGCGGCAGCAGCCACAGCGACAGCGCTGGGGCCCTGTGTAGAAG

CTCCATCCCCCTTGTCTTGTGCTTGCCCTGCGTCCCCAGACTCAGAGATTATCTTAGAAGACCTAGGACTCCAAAA

M F P L K D A E M G A F T F F A S A L P 20 SEQ ID NO:5
 ATG TTT CCC CTG AAG GAC GCT GAA ATG GGA GCC TTT ACC TTC TTT GCC TCG GCT CTG CCA 60
 ↑SEQ ID NO:6→

H D V C G S N G L P L T P N S I K I L G 40
 CAT GAT GTT TGT GGA AGC AAT GGA CTT CCT CTC ACA CCA AAT TCC ATC AAA ATT TTA GGG 120

R F Q I L K T I T H P R L C Q Y V D I S 60
 CGC TTT CAA ATC CTT AAA ACC ATC ACC CAT CCC AGA CTC TGC CAG TAT GTG GAT ATT TCT 180

R G K H E R L V V V A E H C E R S L E D 80
 AGG GGA AAG CAT GAA CGA CTA GTG GTC GTG GCT GAA CAT TGT GAA CGT AGT CTG GAA GAC 240

L L R E R K P V S C S T V L C I A F E V 100
 TTG CTT CGA GAA AGG AAA CCT GTG AGC TGT TCA ACG GTT TTG TGT ATA GCA TTT GAG GTT 300

L Q G L Q Y M N K H G I V H R A L S P H 120
 CTT CAG GGC TTG CAG TAT ATG AAC AAA CAT GGT ATA GTA CAC AGG GCA TTG TCT CCT CAT 360

N I L L D R K G H I K L A K F G L Y H M 140
 AAT ATC CTG TTG GAC CGA AAG GGA CAT ATT AAA TTG GCT AAA TTT GGA CTT TAT CAC ATG 420

T A H G D D V D F P I G Y P S Y L A P E 160
 ACA GCT CAT GGT GAT GAT GTT GAT TTC CCA ATA GGG TAT CCC TCG TAC TTG GCC CCT GAG 480

V I A Q G I F K T T D H M P S K K P L P 180
 GTA ATT GCA CAG GGA ATT TTC AAA ACC ACT GAT CAC ATG CCA AGT AAA AAA CCA TTG CCT 540

S G P K S D V W S L G I I L F E L C V G 200
 TCT GGC CCC AAA TCA GAT GTA TGG TCT CTT GGA ATC ATT TTA TTT GAG CTT TGT GTG GGA 600

R K L F Q S L D I S E R L K F L L T L D 220
 AGA AAA TTA TTT CAG AGC TTG GAT ATT TCT GAA AGA CTA AAA TTT TTG CTT ACT TTG GAT 660

C V D D T L I V L A E E H G C L D I I K 240
 TGT GTA GAT GAC ACT TTA ATA GTT CTG GCT GAA GAG CAT GGT TGT TTG GAC ATT ATA AAG 720

E L P E T V I D L L N K C L T F H P S K 260
 GAG CTT CCT GAA ACT GTG ATA GAT CTT TTG AAT AAG TGC CTT ACC TTC CAT CCT TCT AAG 780

Fig. 13A

R	P	T	P	D	E	L	M	K	D	K	V	F	S	E	V	S	P	L	Y	280
AGG	CCA	ACC	CCA	GAT	GAA	TTA	ATG	AAG	GAC	AAA	GTA	TTC	AGT	GAG	GTA	TCA	CCT	TTA	TAT	840
T	P	F	T	K	P	A	S	L	F	S	S	S	L	R	C	A	D	L	T	300
ACC	CCC	TTT	ACC	AAA	CCT	GCC	AGT	CTG	TTT	TCA	TCT	TCT	CTG	AGA	TGT	GCT	GAT	TTA	ACT	900
L	P	E	D	I	S	Q	L	C	K	D	I	N	N	D	Y	L	A	E	R	320
CTG	CCT	GAG	GAT	ATC	AGT	CAG	TTG	TGT	AAA	GAT	ATA	AAT	AAT	GAT	TAC	CTG	GCA	GAA	AGA	960
S	I	E	E	V	Y	Y	L	W	C	L	A	G	G	D	L	E	K	E	L	340
TCT	ATT	GAA	GAA	GTG	TAT	TAC	CTT	TGG	TGT	TTG	GCT	GGA	GGT	GAC	TTG	GAG	AAA	GAG	CTT	1020
V	N	K	E	I	I	R	S	K	P	P	I	C	T	L	P	N	F	L	F	360
GTC	AA	AAG	GAA	ATC	ATT	CGA	TCC	AAA	CCA	CCT	ATC	TGC	ACA	CTC	CCC	AAT	TTT	CTC	TTT	1080
E	D	G	E	S	F	G	Q	G	R	D	R	S	S	L	L	D	D	T	T	380
GAG	GAT	GGT	GAA	AGC	TTT	GGA	CAA	GGT	CGA	GAT	AGA	AGC	TCG	CTT	TTA	GAT	GAT	ACC	ACT	1140
V	T	L	S	L	C	Q	L	R	N	R	L	K	D	V	G	G	E	A	F	400
GTG	ACA	TTG	TCG	TTA	TGC	CAG	CTA	AGA	AAT	AGA	TTG	AAA	GAT	GTT	GGT	GGA	GAA	GCA	TTT	1200
Y	P	L	L	E	D	D	Q	S	N	L	P	H	S	N	S	N	N	E	L	420
TAC	CCA	TTA	CTT	GAA	GAT	GAC	CAG	TCT	AAT	TTA	CCT	CAT	TCA	AAC	AGC	AAT	AAT	GAG	TTG	1260
S	A	A	A	T	L	P	L	I	I	R	E	K	D	T	E	Y	Q	L	N	440
TCT	GCA	GCT	GCC	ACG	CTC	CCT	TTA	ATC	ATC	AGA	GAG	AAG	GAT	ACA	GAG	TAC	CAA	CTA	AAT	1320
R	I	I	L	F	D	R	L	L	K	A	Y	P	Y	K	K	N	Q	I	W	460
AGA	ATT	ATT	CTC	TTC	GAC	AGG	CTG	CTA	AAG	GCT	TAT	CCA	TAT	AAA	AAA	AAC	CAA	ATC	TGG	1380
K	E	A	R	V	D	I	P	P	L	M	R	G	L	T	W	A	A	L	L	480
AAA	GAA	GCA	AGA	GTT	GAC	ATT	CCT	CCT	CTT	ATG	AGA	GGT	TTA	ACC	TGG	GCT	GCT	CTT	CTG	1440
G	V	E	G	A	I	H	A	K	Y	D	A	I	D	K	D	T	P	I	P	500
GGA	GTT	GAG	GGA	GCT	ATT	CAT	GCC	AAG	TAC	GAT	GCA	ATT	GAT	AAA	GAC	ACT	CCA	ATT	CCT	1500
T	D	R	Q	I	E	V	D	I	P	R	C	H	Q	Y	D	E	L	L	S	520
ACA	GAT	AGA	CAA	ATT	GAA	GTG	GAT	ATT	CCT	CGC	TGT	CAT	CAG	TAC	GAT	GAA	CTG	TTA	TCA	1560
S	P	E	G	H	A	K	F	R	R	V	L	K	A	W	V	V	S	H	P	540
TCA	CCA	GAA	GGT	CAT	GCA	AAA	TTT	AGG	CGT	GTA	TTA	AAA	GCC	TGG	GTA	GTG	TCT	CAT	CCT	1620
D	L	V	Y	W	Q	G	L	D	S	L	C	A	P	F	L	Y	L	N	F	560
GAT	CTT	GTG	TAT	TGG	CAA	GGT	CTT	GAC	TCA	CTT	TGT	GCT	CCA	TTC	CTA	TAT	CTA	AAC	TTC	1680
N	N	E	A	L	A	Y	A	C	M	S	A	F	I	P	K	Y	I	Y	N	580
AAT	AAT	GAA	GCC	TTG	GCT	TAT	GCA	TGT	ATG	TCT	GCT	TTT	ATT	CCC	AAA	TAC	CTG	TAT	AAC	1740
F	F	L	K	D	N	S	H	V	I	Q	E	Y	L	T	V	F	S	Q	M	600
TTC	TTC	TTA	AAA	GAC	AAC	TCA	CAT	GTA	ATA	CAA	GAG	TAT	CTG	ACT	GTC	TTC	TCT	CAG	ATG	1800
I	A	F	H	D	P	F	L	S	N	H	L	N	E	I	G	F	-	P	D	620
ATT	GCA	TTT	CAT	GAT	CCA	GAG	CTG	AGT	AAT	CAT	CTC	AAT	GAG	ATT	GGT	TTC	ATT	CCA	GAT	1860
L	Y	A	I	P	W	F	L	T	M	F	T	H	V	F	P	L	H	K	I	640
CTC	TAT	GCC	ATC	CCT	TGG	TTT	CTT	ACC	ATG	TTT	ACT	CAT	GTA	TTT	CCA	CTA	CAC	AAA	ATT	1920

Fig. 13B

F H L W D T L L L G N S S F P F C I G V 660
 TTC CAC CTC TGG GAT ACC TTA CTA CTT GGG AAT TCC TCT TTC CCA TTC TGT ATT GGA GTA 1980
 A I L Q Q L R D R L L A N G F N E C I L 680
 GCA ATT CTT CAG CAG CTG CGG GAC CGG CTT TTG GCT AAT GGC TTT AAT GAG TGT ATT CTT 2000
 L F S D L P E I D I E R C V R E S I N L 700
 CTC TTC TCC GAT TTA CCA GAA ATT GAC ATT GAA CGC TGT GTG AGA GAA TCT ATC AAC CTG 2100
 F C W T P K S A T Y R Q H A Q P P K P S 720
 TTT TGT TGG ACT CCT AAA AGT GCT ACT TAC AGA CAG CAT GCT CAA CCT CCA AAG CCA TCT 2160
 S D S S G G R S S A P Y F S A E C P D P 740
 TCT GAC AGC AGT GGA GGC AGA AGT TCG GCA CCT TAT TTC TCT GCT GAG TGT CCA GAT CCT 2220
 P K T D L S R E S I P L N D L K S E V S 760
 CCA AAG ACA GAT CTG TCA AGA GAA TCC ATC CCA TTA AAT GAC CTG AAG TCA GAA GTA TCA 2280
 P R I S A E D L I D L C E L T V T G H F 780
 CCA CGG ATT TCA CCA GAG GAC CTG ATT GAC TTG TGT GAG CTC ACA GTG ACA GGC CAC TTC 2340
 K T P S K K T K S S K P K L L V V D I R 800
 AAA ACA CCC AGC AAG AAA ACA AAG TCC AGT AAA CCA AAG CTC CTG GTG GTT GAC ATC CGG 2400
 N S E D F I R G H I S G S I N I P F J A 820
 AAT AGT GAA GAC TTT ATT CGT GGT CAC ATT TCA GGA AGC ATC AAC ATT CCA TTC AGT GCT 2460
 A F T A E G E L T Q G P Y T A M L Q N F 840
 GCC TTC ACT GCA GAA GCG GAG CTT ACC CAG GGC CCT TAC ACT GCT ATG CTC CAG AAC TTC 2520
 K G K V I V I V G H V A K H T A E F A A 860
 AAA GGG AAG GTC ATT GTC ATC GTG GGG CAT GTG GCA AAA CAC ACA GCT GAG TTT GCA GCT 2580
 H L V K M K Y P R I C I L D G G I N K I 880
 CAC CTT GTG AAG ATG AAA TAT CCA AGA ATC TGT ATT CTA GAT GGT GGC ATT AAT AAA ATA 2640
 K P T G L L T I P S P Q I * 894
 AAG CCA ACA GGC CTC CTC ACC ATC CCA TCT CCT CAA ATA TGA 2682
 †SEQ ID NO:6†
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 TTCAGTTATCCATGCAAGAACTTGACTCTACATGTATTGCTGAAAGAATTTTCTTAACAGTGAAATCTGATCATATAT
 TTTTACCACACTGCCACATAAAGCCCAAGAAATTCAGCTGACAAGACAGATTTAGCATTATCAAGAAATC ATTTGCC
 CTGAAAAAGCTGTCTCCATTGTAAGTGAACAGACAGTCCTGTGATTGTGTTATTTAGAAACATACACTGAATGTGGGC
 TGAAATCATCATCTTTCATAATGAAAACCTGAGAACTATTACAATGCATTCCTTATAAATAAATGCTACATTTAGTA
 ACTCATTTACCCAAACAAGAAAGATCTGTGTGTGTGTGTATAGGAAGTGGAGTTTATCCCCATTGC:..AAACTT
 AATACTTACTCCCAGAAAAATGAAATTTAGAAACCATTTATATTTGATAGAATATTTGGTCAGTTCCTGTAGCAAAGAC
 GAATGGCTTAAACAAATTTTCTAGTTTCTTTATCACATGAAAGTCTGTACAGTCAGTCCAGGGCTAGTCTACTGGTTTC

Fig. 13C

CTGATCATTAAAGAACTCATTACCTTCTCTCATTGCTTTACAAACCTCAATATGTGGCATCCATCTCATGGATGAAAATG
GCTCCTCAGCTTCTACCATCACATCTGCTATCTAGAAGGAAGAGAATGAGGGAAGGAGGGAGGGGATGAAGAGAAAAGA
AGGAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 13D

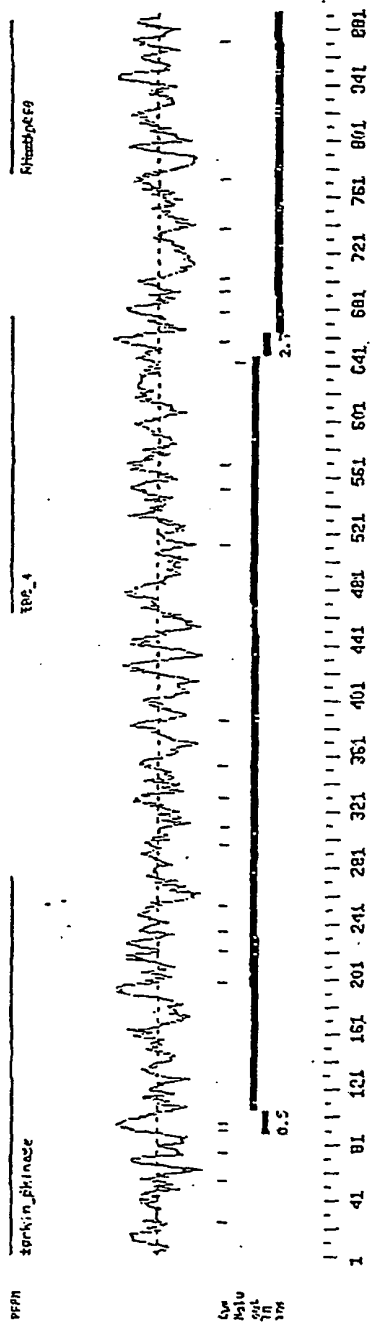


Fig. 14

12/16

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18431 43 QILKTITHPRLCQYVDISRGKHERLVVVAEH CER-SLEDLLRERKPV 88

sekeakkialQilrGleYlHsngivHRDLKpeNILLdengtvKiaDFGLA
s +++ia ++l+Gl+Y+ +givHR L p NILLd++g++K+a FGL
18431 89 SCSTVLCIAFEVLQGLQYMNKHGIVHRALSPHNILLDRKGHIKLAFLGLY 138

rll...eklttfvGTPwYmmAPEvi.....leg.rgysskvDv
++ G p Y APEvi ++ +++++ + + + + ++k+Dv
18431 139 HMTahgDDVDPIGYSYL-APEVIAqgikfkttdhmpSKKpLPSGPKSDV 187

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WSlG+il+El+ g++lf+++d ++ l +
18431 188 WSLGIILFELCVGRKLFQSLD-----ISERLKFLTL 219

ridpleelfrikkr.rlpplsncSeelkdLlkkcLnkDPskRpGsatake
++ ++ ++ ++e+++dLl+kcL++ PskRp t e
18431 220 DCVDDTLIVLAEHqCLDIKELPETVIDLLNKCLTFHPSKRP---TPDE 266

ilnhpwf<-*
+++++ f
18431 267 LMKDKVF 273

```

Fig. 15

```

*->vrqgvpslRgkVWklilgaqelnnciltdnfkgldlfglvpvllla SEQ ID NO:18
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dkdeYeellnknkektvqdqneKssvgirrlidyVEAVEKHPLSDNDKTK
Y+++++ ++ +t
18431 487 -HAKYDAIDKOTPIPT----- 501

GSLekgsdekalklredldkIekDlsRTfpdeiffqtrlaeqqlkkdqdl
+ +Ie D+ R+ + +++
18431 502 -----DRQIEVDIPRCHQYDELL----- 519

daydkDEfddeddkneppsikqlrrlLvaYswknpqehlgyvQGMnvils
+p+++++ rr+L a ++ +p+ l Y QG + +
18431 520 -----SSPEGHAKFRRVLKAWVVSHPD--LVYWQGLDSLCA 553

pLLlf.lkhgvdldideeqAFwclvkLmdnylpqkyflndls.glnedl
p+L++++ +e A++c +++++ +yl + +fl+d s+ ++e l
18431 554 PFLYLnFN-----NEALAYACMSAFIPKYLYN--FFLKDNShVIOEYL 594

rvLdslvkeslPeLyshlkkkenktgsgkKknllaldltllifafpwlLt
v++ + + +PeL++hl+ + +++++a+pwlLt
18431 595 TVFSQMIAFHDPeLSNHLNEI-----GFIPDLYAIPWFLT 629

lFarelPleivlrIwDilftyYlgshflifvalAiLklkksklkh<-*
F+ ++Pl +++++wD l++ +s+f++ +++AiL++l++ ll+
18431 630 MFTHVFPLHKIFHLWDTLLLG--NSSFPFCIGVAILQQLRDRLLAN 673

```

Fig. 16

Query: 459 IWKEARVDIPPLMRGLTWAALLGVEGAIHAKYDAIDKDT-----PIPTDRQIEVDIPRC 512
 I +E +D+PP +RG W LL V + +Y +D P P DRQ+EVDIPRC
 Sbjct: 1 IQRETNIDVPPTLRGEVWGCLLRVPSPARTRYALLDHAVHHTAAKPTPHDRQLEVDIPRC 60 SEQ ID NO:19

Query: 513 HQYDELLSSPEGHAKFRRLKAWVVS--PDLVYWGQLDSLCAFLYLNFNEALAYACM 570
 HQY LL+SP G A+ RR+LKAW + + P+ VYWQGLDSLCAFL +N +EALA+A +
 Sbjct: 61 HQYHPLNLSPSGSAQLRRILKAWQIVYLRPEHVYWGQLDSLCAFLTVNRRDEALAFACL 120

Query: 571 SAFIPKYLYNFFLKDNNSHVIQEYLTVFSQMIAFHDPELSNNHLNEIGFIPD 620
 +AF+ +Y++ F+LKDNS VI+EYL F + A+HDP L HL GF P+
 Sbjct: 121 NAFVNRYYIHWFYLKDNSEVIKEYLGKFYHLTAYHDPILLYQHLKINGFDPE 170

Fig. 17

Query: 27 NGLPLTPNSIKILGRFQILKTITHPRLCQYVDISRGKHER--LVVVAEHCERSLEDLLRE 84
 NGLPLTP + ++LGRF L+ + H LCQY+ RGKHER +VV EH +LED +
 Sbjct: 1 NGLPLTPPAQMLGRFPYLQELQHDHLCQYLHFIRGKHERDLTIVVMEHYGMNLEDYAKR 60 SEQ ID NO:20

Query: 85 RKPVSCSTVLCIAFEVLQGLQYMNKHGIVHRALSPHNILL----DRKGHIKLAFLGLYHM 140
 P + +++ G+ Y+++H IVH L P++I + +RK +KL +GL+HM
 Sbjct: 61 HPPKDEAQNNNFYQIACGINYLHRHHIVHNLHPNHIYITDDGNRKLSVKLFNYGLHMH 120

Query: 141 TAHGDDVDVDFPIGYPSYLAPEVIAQGIKTTDMMXXXXXXXXXXXXVWSLGIILFELCVG 200
 T +G FPIG Y+APE I D++ DVW LG I+ ++ +G
 Sbjct: 121 TNYCKYTPFPIGNGRYMAPE----RILNDNDNLFAATYQS-----DVWELGFIMLQIYLG 171

Query: 201 RKL 203
 +L
 Sbjct: 172 IEL 174

Fig. 18

Query: 322 IEEVYYLWCLAGGDLEKELVNKEIIRSKPPICTLPNLFEDGESFGQGRXXXXXXXXXXXX 381
 + ++Y+LW LAGGD++ EL + +IRS+ PI LP + G S GR
 Sbjct: 100 LSQIYHLWQLAGGDVQAEKKEGLIRSEAPILGLPQIVRLSGASVCPGRSQAQLMDDRNV 159 SEQ ID NO:21

Query: 382 XXXXCQLRNRKLDVGEAFYPLLEDDQSNLPHSNSNNELSAATLPLIIREKDEYQNLNR 441
 L RL + ++PLL + P ++ EL LPL+IREKD EYQ R
 Sbjct: 160 PLRLKALLQRLSGLPAAVYFPLHSPR--FP-AHFARELQE---LPLVIREKDIEYQFQR 213

Query: 442 IILFDRLLKAYPYKKNQ 458
 + LF RLL+ YP+ Q
 Sbjct: 214 VRLFARLLQGYPHTAEQ 230

Fig. 19

Query: 207 LDISERLKFLLTLDCVDDTLIVLAEHGCGLDIKELPETVIDLLNKCLTFHPSKRPTPDE 266
 L +S ++ +L + L +A EH C + ++ ++ LL CL+ P +RP P E
 Sbjct: 4 LKLSNVVRKILAFGKSNGALEKIAREHQCHERYVQMDQRLRQLLESCLSVLPKRRPLPGE 63 SEQ ID NO:22

Query: 267 LMKDKVFSEV 276
 L++ +F EV
 Sbjct: 64 LLEHPIFEV 73

Fig. 20

Query: 636 PLHKIFHLW 644
 PL +I+HLW
 Sbjct: 99 PLSQIYHLW 107 SEQ ID NO:23

Fig. 21

Query: 621 LYAIPWFLTMFTHVFPLHKIFHLWDTLNLSGSSFPFCIGVAILQQLRDRLL-ANGFNECI 679
 LYA WFLT+F PL + +WD S F + +A+L+ ++ LL A+ F E +
 Sbjct: 1 LYAFQWFLTLFARELPLETVLRIWDCFFYEGSKILFRVALALLKMHKEELLQADDFEEML 60 SEQ ID NO:24

Query: 680 -LLES DLP-----EIDIERCVRESINL 700
 L + LP E D R + E+ N+
 Sbjct: 61 EFLQNMLPKRYRSEEDARRLLEEACNI 87

Fig. 22

Query: 72 EHCERSLEDLLRERKPVSCSTV---LCIAFEVLQGLQYM---NKHGIVHRALSPHNILL 124
 E E+ + + E+K S V + IA+++ +GL+Y+ NK I+HR L P NILL
 Sbjct: 136 EMMEKQLKQSMSEKMEEMSWSQMLMKIAYQIAKGLEYLHSSKNQNIHRDLKPENILL 195 SEQ ID NO:25

Query: 125 DR----KGH-----IKLAKFGLYHM 140
 D KG +K+A FGL M
 Sbjct: 196 DNNMVAKG DSEIKVVKIADFG LARM 220

Fig. 23

Query: 152 GYPSYLAPEVIAQGIFKTTDHMXXXXXXXXXXXXXDVWSLGIILFELCVGRKLF--QSLDI 209
 G PSY+ + + + + DVWS G+IL+EL G+ F S ++
 Sbjct: 245 GTPSYV--KYVGTRWYMAPEVLMGSSYGQYSEKSDVWSFGVILYELTGKPPFFPGSSEV 302 SEQ ID NO:26

Query: 210 SE-RLKFLTLDCVDDTLIVLAEHGC LDIKE---LP----ETVIDLLNKCLTFHPSKR 261
 ++ ++ ++ V + + + KE P E V DL+ KC P KR
 Sbjct: 303 NDSQMNEIMKETMVKSAEYEMPMKMPPESSKESMSCPSMSSEAVKDLIKKCWQKQDPEKR 362

Query: 262 PTPDELMKD KVFSEV 276
 PT +++++ E+
 Sbjct: 363 PTFAQVVEELSAHEI 377

Fig. 24

Query: 740 PPKT-DLSRESIPLNDLKSEVSPRISAEDLIDLCELTVTGHFXXXXXXXXXXXXXXXXLLVVD 798
 PP+ D+ + L L+ E PRISA+D+ L + L ++D
 Sbjct: 29 PPQALDIGVADVELKHLQEQCPRISAKDVQFLD-----NSPAELALID 73 SEQ ID NO:27

Query: 799 IRNSED FIRGHISGSINIPFSAFTAEGELTQGPYTAMLQNFKGKVIVIVGHVAKHTAEF 858
 +R+ +F R H+ SINIPF+ E L + +GK++V V ++ +H+ E
 Sbjct: 74 LRSVVEFGRVHVP HSINIPFATVQLGEQRLEALQVPQLEAQLRGKIVVCVSNIHQHSVEV 133

Query: 859 AAHLVKMK 866
 L ++K
 Sbjct: 134 GHPLAQLK 141

Fig. 25

Query: 693 CVRESINLFCWTPK SATYRQHA---QPPKPXXXXXXXXXXAPYFSAECPDPPKTDL--- 745
 CV ES ++ TPKS T+RQHA QPP+ +CP D+
 Sbjct: 3 CVLESQKMYEATPKSITHRQHALRLQPPQALDIGVADVELKHLQEQCPRISAKDVQFLL 62 SEQ ID NO:28

Query: 746 --SRESIPLNDLKSEVS-PRISAEDLIDLCELT V 776
 S + L DL+S V R+ I++ TV
 Sbjct: 63 DNSPAELALIDLRSVVEFGRVHVPHSINIPFATV 96

Fig. 26

Query: 856 AEFAAHLVKMKYPRICILDGGIN---KIKPTGLLT 887
 ++F+ LV R CIL G N I+P L++
 Sbjct: 152 SQFSHFLVACGVQRTCILHKGFNVLHSIEPNILIS 186 SEQ ID NO:29

Fig. 27

Query: 506 EVDIPRCHQYDELLSSPEGHAKFRRLKAWVVSHPD--LVYWQGLDSLCAFLYLNFNNE 563
 + DI C +Y+ P + + + L+ + V +P ++ + G APF YL
 Sbjct: 336 DTDIGGCFEYNTF-PPPGKYRGLGLEEYAVFYPPNGVIPFHGFCMYAAPFCYLYHEPS 394 SEQ ID NO:30

Query: 564 ALAYACMSAFIPKYLYNFFLKDNSHV--IQEYLTVFSQMIAFHDPELS NHLNEIGFIPDL 621
 L Y +I +Y + N+H I +F +++ ++P+L H EIG P
 Sbjct: 395 KLYYTFREFYI-RYCHRLHTI-NTHPQGIVSLCLLFEKLLQTYEPQLWYHFREIGAQPLR 452

Query: 622 YAIPWFLTMFTHVFPLHKIFHLWDTLLLGNS 652
 + W + F+ P ++ LWD +L NS
 Sbjct: 453 ISFKWMMRAFSGHLPPDQLLLLWDRILGYNS 483

Fig. 28

Query: 77 SLEDLLRERKPVSCSTVLC--IAFEVLQGLQYMNKHGIVHRALSPHNILLDRKGHIKLA 134
 S LLR P S L I F ++GL Y++++G +HR++ +IL+ G + L+
 Sbjct: 5 SASQLLRITYFPEGMSETLIRNILFGAVRGLNVLHQNGCIHRSIKASHILISGDGLVTL 64 SEQ ID NO:31

Query: 135 FG-LYHMTAHGDD---VDFP---IGYPSYLAPEVIAQGIKTTDHMXXXXXXXXXXXXD 186
 L+ + HG DFP +L+PE++ Q + H D
 Sbjct: 65 LSHLHSLVKHGQRHRAVDYDFPQFSTSVQPWLSPELLRQDL-----H-----GYNVKSD 112

Query: 187 VWSLGIILFELCVGRKLFQSLDISERL 213
 ++S+GI EL G+ FQ + ++ L
 Sbjct: 113 IYSVGITACELASGVPPFQDMHRTQML 139

Fig. 29

Query: 240 KLPETVIDLLNKCLTFHPSKRTPDELMKDKVFSEV 276
 K L+ CL P KRP+ L+ F ++
 Sbjct: 198 KTFSPAFFSLVQLCLQDPEKRPSSASSLLSHVFFKQM 234 SEQ ID NO:32

Fig. 30

Query: 596 VFSQMIAFHDPELSNHLNEIGFIPDLYAIPWFLTMFTHVFPLHKIFHLWDTL LLGNSSFP 655
+F ++ +HDPEL NHL+ PD+Y + WF ++F LWD + F
Sbjct: 18 LFRLLQLQYHDPELCNHLDTKKCTPDMYTLNWFGLFASCCSTEVCHALWDLYIQQADPFM 77 SEQ ID NO:33

Query: 656 -FCIGVAILQQLRDRLL---ANGFNECILLFSDLP-EIDIE 691
F + + IL ++ +L ++ E I ++P +++IE
Sbjct: 78 VFFLALIILINAKEEILQMKSDSKEEVIKFLENMPCQLNIE 118

Fig. 31

Query: 796 VVDIRNSED FIRGHISGSINI 816
+VD R +E + GH+S + ++
Sbjct: 194 IVDCRPAEQYNAGHLSTAFHL 214 SEQ ID NO:34

Fig. 32

Query: 112 IVHRALSPHNILLDRKGHIKLAKEGLYHMTAHGDDVD--FPIGYP--SYLAPEVIAQGIF 167
++HR + P +IL+ ++G KLA F + +D + FP Y + P + +
Sbjct: 1 VIHRNICPESILITKRGSWKLAGFDFCVSAQNPNQENYFPCHYEWDPRIPPLPLQPNLD 60 SEQ ID NO:35

Query: 168 KTTDHMXXXXXXXXXXXXVWSLGIILFELCVGRKLFQSLDISERLKFLTLDCVDDTLI 227
D++SLG +++ + G K +D + ++ + +TL
Sbjct: 61 YLAPEYVTSSTCTVGSASDMFSLGCLIIYIYNGGKPL--IDANNDEYKSNYNKYMNTLN 118

Query: 228 VLAEHGCCLDIKELPETVIDLLNKCLTFHPSKRPTPDELMKDKVF 273
L H ++ + PE + + L + L+ P+ RPT EL K F
Sbjct: 119 SLT--HESMNNLP--PENLKESLKRMLSMDPTVRPTAQELTLIKYF 160

Fig. 33

Query: 744 DLSRESIPLNDLKSEVSPRI--SAEDL-IDLCE 773
DL + P D+KS + P + + ED I +C+
Sbjct: 273 DLLLQKTPPEDIKSNILPMLYYAFEDSDIQC 305 SEQ ID NO:36

Fig. 34

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